

nature**28 June 1979**

Problems of civil service pay comparisons

Last Friday, many tens of thousands of scientists and technologists, members of the British Civil Service, went on strike for a day. More action — withdrawal of goodwill and selective strikes — is promised by the Institution of Professional Civil Servants (IPCS), which represents about 90% of the civil servants in the relevant categories. For the majority of those who obeyed the strike call, a day away from work probably provided a welcome opportunity to catch up with some reading, secure in the knowledge that the nation's scientific and technological future is hardly put in jeopardy by a day's absence. But IPCS does have muscle; although its officials were at pains to point out that the day was one of protest not disruption, highly visible disruption there certainly was in the area of air traffic control.

Few of those involved in the strike action can have gone as far as this before — what caused the normally moderate and restrained IPCS to use its ultimate weapon? The answer, put simply, is pay, and in particular comparisons between the pay of civil servants and their counterparts outside the service. IPCS negotiates with Civil Service Department (CSD) officials (ultimately with the Minister of State for the Civil Service Department, Mr Paul Channon) over salary. There was a time when the pay for each of the many grades of scientist and technologist was established by a process of 'pay research' — painstaking comparisons were made between industry, commerce, the professions and the Civil Service in order to decide a fair level of remuneration. On the whole these comparisons were based on median salaries outside the Civil Service, but there was one significant exception — the Professional and Technological group of employees, (not the scientists), numbering about 40,000. Traditionally their pay settlements had been at well above the median of outside salaries on the basis that many outsiders, in fields such as architecture and surveying, were in business by themselves and not included in the comparisons, that there were heavy responsibilities and demands of very high quality on civil servants and so on.

Severe pay restraint from 1975 onwards meant an abandonment of pay research at a time when Civil Service salaries looked good — even better if pensions' provisions and stability of employment were allowed for. Since that time there is little doubt that the scientists and technologists in the Civil Service have lost their edge on salaries, and as pay research has gradually come back into action the extent of the increases necessary to bring civil servants back into line has become apparent. The Professional and Technological grade has been offered comparability with the median (raises of between 15% and 22%) rather than comparability with a figure well above the median — CSD rejected IPCS demands that the former grounds for favourable treatment be retained.

It had already been agreed that the Science Group (around 17,000 people) should not go back into pay research comparisons until 1980, and last year CSD declared in writing that it was 'context to accept' IPCS's proposal that for 1979 scientists' pay should be linked to that of administrators. But when administrators got an increase of 25% or more, CSD were unwilling to match the salaries for scientists. It has since done so (on 15 June) but only on condition that future pay for scientists should be based on pay research, even if this should lead to salary reductions next year, and that P & T grades would also abide by pay research (at the median, presumably).

What is behind all this? On the P & T side it is clear that the Government is unconvinced that settlements above the median are now called for, believing that they were based on a small number of special cases. On the Science side, there seem to be unambiguous warnings that the 1980 recommendations from pay research are not going to be very good news; one CSD official commented that there was a possibility that pay research, although not for implementation until 1980, 'might be relevant to the course we should take in 1979'. In other words, brace yourselves for a nasty shock.

There is a real problem here. The heart of the Scientific Civil Service is the two thousand or so Principal Scientific Officers. This is a level up to which many scientists will be moved in their late thirties but out of which relatively few will be promoted. So most PSOs will work at this grade for twenty years or more. Scientists outside the Civil Service are unlikely to be in anything like the same situation, so pay research comparisons at the PSO level (and maybe even just below it) are difficult, to say the least. As a consequence this may lead to depressed salaries.

That scientists and technologists need a pay boost is beyond doubt; the steady trickle of young computer people, for example, out of the service and into industry has to be stopped. But on the other hand salaries which look so good in comparison with industry when that industry is denuded of good people undoubtedly jeopardise Britain's industrial future. So some fine balancing has to be done.

What is in danger of being lost, however, in the heightened temperature of a strike, is the long-term need for a very serious review of the use that the nation should be making of its older scientists. It keeps them in laboratories well beyond the time many of them can (by their own admission) make an adequate contribution; their way into other parts of the Civil Service is barred, despite the benefits that numerate, scientific thinking might bring. As part of any settlement, IPCS and CSD ought to agree to look very carefully at the problem of the Principal Scientific Officer. □



US seeks to 'rationalise' health and safety regulation in the interest of profits

The role of science in the regulatory process was a central theme in a meeting on science policy held last week by AAAS.

David Dickson reports

THE Carter administration is launching a concerted effort to rationalise its environmental and health regulatory mechanisms in a way that will ensure these mechanisms do not sap the vitality of the private sector. According to Mr Bowman Cutter, head of budget affairs in the Office of Management and Budget, the administration's overall aim is "to promote a structure in which decisions [about future investment] can be made rationally."

Precise details of the administration's proposals are expected to be announced shortly. Various alternatives are included among a list of possible decisions now facing President Carter on ways to stimulate industrial innovation, the results of a year-long study by the Department of Commerce.

However the general philosophy of the administration's approach was outlined by three of its principal architects — Mr Cutter, Dr Frank Press, director of the Office of Science and Technology Policy and Dr Jordan Baruch, assistant secretary of commerce — at a meeting on federal research and development policy held last week by the American Association for the Advancement of Science.

From this it emerged that a major emphasis will be placed not on ways in which direct intervention can aid innovation in private firms, but on ways of stabilising and "rationalising" the decision-making environment. And where the Commerce Department's report has provided the rationale, OMB has been studying institutional changes that will help make this possible, and OSTP looking at ways in which science and the scientific community can contribute.

Indeed having done much to set basic research funding on its feet in the first two Carter budgets — Mr Cutter told the AAAS meeting that basic science could expect a further modest amount of real growth in the 1981 budget — the role of science in the regulatory process has now become a central concern to OSTP.

Many companies blame excessive regulation as one reason for the poor performance of the US economy. "Industry has been compelled to spend more and more of its research dollars to comply with environmental, health and safety regulations — and to move away from longer-term efforts aimed at major scientific advances" is a typical complaint from Rawleigh Warne Jr., the chairman of Mobil Oil.

The problem facing the administration is that much of this regulation has been mandated by Congress. It is therefore suggesting, not less regulation as such, but that a distinction be made between necessary and "unnecessary" regulation — a distinction whose validity is questioned by many trade unionists — and that the regulatory burden be rationalised by cutting out the latter.

"Society has made decisions to repurchase clean air and water and to protect the health of workers," Dr Baruch, who was responsible for co-ordinating the Commerce Department study, told the AAAS meeting. "It is imperative that we do not sacrifice the goals of those decisions, but ask how they can be made compatible with the desire to stimulate innovation."

Dr Cutter described the significant impact such regulatory decisions have had on the political landscape. "Prior to the 1960s regulation was largely economic. But subsequently a very new form of regulation has begun to be developed linked to central social objectives," Mr Cutter said.

Similar points were made by Dr Press (in a speech delivered by OSTP associate director Dr Phil Smith), who criticised current regulatory efforts as being "highly-segmented, wide-ranging in impact, economically important, highly politicised, very aggressive, relatively independent, and almost totally uncoordinated."

Specific characteristics, he said, included that existence of distinct regulatory regimes, tough legislative mandates, single priority objectives with less consideration to other impacts (especially costs), the delegation of authority to separate agencies, and "a lack of any mechanism for weighing the overall impact of the sum of the separate programmes."

A major goal of the present administration, which had already implemented various activities to remedy this situation, was "improved care and rationalism, in both substance and process" he said. One area in need of improvement was the uniform application of scientific principles in the regulatory process. Another was the possible use of neutral experts to help "fence in" controversial areas so that debates on regulation "can be confined to legitimate differences in values."

University researchers could play an



"We figured the more regulators there were, the less they'd bother us — and it works!"

important role in these type of fields, he said, for example through campus based research centres. And there was no need for regulatory programmes to inhibit socially desirable innovations.

Both Dr Press and Mr Cutter admitted that rationalising the regulatory process was unlikely to be an easy task. Last year, for example, OMB had brought together seven agencies to compare their various programmes on toxic substances. But Mr Cutter admitted that the results of the exercise had been "not too successful".

This, he said, was partly due to substantial differences in objectives between the various bodies, with regulatory agencies, for example, claiming that the need to fulfil legal mandates was a principal reason for doing research, while basic research agencies gave highest priority to the expansion of knowledge.

"We will have to continue work on this. Research on the impact of regulation must increase; but before we do this, the federal government will have to get its house in order so that we know what we are spending money on and why we are doing it," Mr Cutter said.

Both speakers also agreed that the current position of "social regulation" was similar to that of economic regulation prior to the establishment of OMB by the Budget and Accounting Act of 1921, which for the first time brought the budgets of the various federal agencies together under direct presidential control.

Sceptics point out, however, that this act provided Presidents Harding and Coolidge with both the means and the authority to slash federal spending and keep it down, with total expenditures dropping from \$5.1 billion in 1921 to \$3.4 billion in 1922, and remaining low until the end of the decade. They are hoping that history will not repeat itself too closely. □

Carter aims at 20% solar energy

PRESIDENT Carter last week unveiled a package of proposed legislative measures designed, he said, towards meeting a national goal of 20% of US energy needs coming from solar and renewable resources by the end of the century.

Among the proposals are the setting up of a \$100 million solar energy bank to subsidize the interest on loans and mortgages made to home-owners and businessmen to install solar energy devices; extensive tax credits for solar installations; and the shift in emphasis from demonstration programmes of high-cost centralised solar electric technologies to "those systems which hold wider potential to displace the use of oil and natural gas".

As a result of these new programmes, the total commitment of the federal government in 1980 to solar energy will be more than \$1 billion, "a significant milestone for our country", the President said in a message to the Congress listing his proposals.

However the President's decision to fund some of the developments, including in particular the solar energy bank, from a tax he is proposing on the "windfall profits" expected by oil companies as a result of recent price increases, has been attacked by environmentalists as a political move to gather support for the proposed tax, and thus putting the solar efforts in jeopardy.

In deciding on an ambitious target of 20% solar energy by 2000 — of which one third may come from solar heating, one third from solar cells, and the rest from sources such as hydropower, windpower and the conversion of waste products into energy — the President had had presented to him a range of options, some of which placed the target much higher.

But Dennis Hayes, a research fellow at the Worldwatch Institute in Washington and a leading advocate of solar power, told a House subcommittee the previous week that the cost of the higher options, which administration officials had estimated to cost \$113 billion to reach a solar contribution of one-third of the US energy needs by 2000, had been substantially overestimated, and that in an era of fiscal frugality the high estimate had been a "kiss of death" to more ambitious plans.

The main proposals in the President's programme are:

- a 20% tax credit up to \$2,000 on new houses built to maximise the use of the sun's energy through "passive" designs
- a tax credit for commercial and multi family buildings of \$20 per million Btu saved beyond energy standards for large buildings
- increasing from 10 to 25% the tax credit for solar equipment designed to provide heat for industrial and

agricultural purposes such as drying crops

- a 15% tax credit for the purchase and installation of air-tight woodburning stoves in principal residences

- a permanent exemption from the 4-cents-a-gallon federal gasoline tax on gasohol, a mixture of 90% gasoline and 10% alcohol.

The response of environmentalist groups to the President's proposal however has been little more than lukewarm. Many of his suggestions, they point out, are already being pursued by individual legislators, while linking the solar bank to the energy trust fund, itself a highly controversial proposal in Congress, is, they claim, an unnecessarily risky move.

Many also doubt whether the amount of money which the President proposes to spend on solar energy is sufficient to head toward the goal by 2000 that he has set the nation, especially when compared to the amount of money that continues to be spent on the development of other energy resources such as nuclear energy.

"The proposed programme is a big disappointment to us; the President is talking about a minimal 5% spending on solar energy compared to other energy sources to reach an ambitious 20% goal," said Herb Epstein of the Solar Lobby, adding that his and other groups would seek to have the financial support and tax credits substantially increased as the proposals passed through Congress. □



Here comes the sun: research on solar cells using a fresnel lens at the Sandia Laboratory

Senate vetoes plan for Third World research agency

CONSERVATIVES in the US Senate, quoting the need to restrain both public spending and the growth of the federal bureaucracy, have rejected President Carter's proposal to establish a new institute for scientific and technological research related to the needs of developing countries.

The Senate's action is not necessarily fatal to the plans for the institute, provisionally known as the Institute for Scientific and Technical Cooperation (ISTC). The House of Representatives last month defeated a similar move to kill the institute; and the legislation establishing it may therefore well be restored when Senate and House conferees meet next week to resolve differences before the relevant parts of the foreign aid bill become law.

However the Senate's decision took many of the institute's supporters by surprise, being described in terms ranging from "shock" to "disaster". "The decision is certainly very disappointing, and has effectively derailed the ISTC at the present time, although we hope that it will not turn out to be decisive," Dr Ralph Smuckler, head of the institute's planning office, told *Nature* last week.

One effect has been to cast a shadow over US preparations for the United Nations Conference on Science and Technology for Development, at which ISTC is planned to be a centrepiece in the US presentation. There is some concern that, in an attempt to appease the critics, the administration might be tempted to water down the proposal and tie it more closely to existing programmes and agencies — a move which, some fear, could seriously diminish the credibility of the institute in the eyes of many Third World countries.

It has largely been in response to inadequacies in current aid programmes that the idea of an institute specifically devoted to scientific research on Third World issues has been discussed in Washington and elsewhere since the mid-1960s, most recently in a report from the Brookings Institute.

Following these discussions, President Carter announced in a speech in Venezuela last March that he was proposing setting up a new institute with two main aims: to focus scientific effort on specific problems facing Third World countries, in areas such as health, medicine and agriculture, as well as "global" problems such as energy and the environment; and to assist developing countries in establishing their own research capabilities as a necessary step towards modernisation.

The principle of working towards these

two objectives has received wide support from virtually every sector of the US scientific and political community. The main point of dispute, however, has been over appropriate procedures, and specifically the extent to which such activities should be tied to the policy directions of other institutions.

The administration has argued publicly that although ISTC will be conceived as part of an overall development assistance strategy, the existence of a council of advisers to the ISTC's director will provide sufficient autonomy; and, privately, that anything giving greater Third World involvement in decision-making — along the lines, for example, of Canada's International Development Research Center — would fail to gain Congressional support.

During last week's debate, the Senate rejected an amendment proposed by Senator Adlai Stevenson to increase the institute's autonomy by making the directors responsible to a board of directors. Although this proposal has been widely supported in the academic community, the administration claimed that it would weaken the links to other development efforts.

Supporters of the bill had spent considerable time putting their case against the Stevenson proposal. In doing so, it turned out that they had paid insufficient attention to the threat from the other direction, namely a growing conservative constituency in the Congress pledged to cut public expenditure and the federal bureaucracy.

These were the forces that provided support for an amendment from Senator Dennis Deoncini, a Democrat from Arizona, proposing that the legislation setting up the proposed ISTC be removed entirely from the International Development Assistance Act for 1980.

"At a time when the American people are themselves struggling to make ends meet, because of spiralling inflation and the beginnings of what promises to be a substantial recession, we can ill afford another well-intentioned but expensive agency to study and coordinate the problems which are all too depressingly familiar," Senator Deoncini said.

Although the funding requests associated with the institute were not great — the administration is initially asking for an additional \$25 million to support the institute's activities in the first year, most of its budget resulting from the transfer of research projects under way in the Agency for International Development (AID) — it would subsequently devour tax dollars at an ever-increasing rate, he said. If the new agency was a response to shortcomings in AID, then improvements should be made in AID, rather than creating a "whole new bureaucracy".

Senator Deoncini was joined by Senator Robert Dole of Kansas, who referred to reports appearing in the

national press that the United Nations currently holds a substantial amount of operating funds in low-interest bank deposits. "We do not need to solve another social problem by throwing more money at it — particularly at a time when similarly destined money is presently being wasted," he said.

Supporters of the administration proposal replied to such charges by claiming that the ISTC would considerably increase the effectiveness of US aid efforts, but in the end their arguments failed to carry sufficient weight; and the amendment rejecting the institute from the aid bill was agreed by 58 votes to 42 — a margin which is said to have taken even the amendment's supporters by surprise.

"It was essentially a conservative vote, with people in the middle whose support we had previously been counting upon

arguing that the line on spending had to be drawn somewhere, and choosing this as the issue on which to do it," one administration official told *Nature* last week.

The precise fate of ISTC will not be known until representatives of the House and the Senate meet in the near future to negotiate over their differences on the foreign aid bill, in order to come up with a form that will be acceptable to both sides and can therefore be signed into law. The hope is that the conferees will agree to keep the institute in, possibly in return for cuts elsewhere.

But the restoration of funds is by no means certain. As one Washington lobbyist said last week: "Congress seems to have gone crazy this year in its desire to cut the budget; and the things that are easiest to cut are the things that are related to overseas." □



Mutual applause: Carter and Brezhnev after the SALT treaty

SALT warning on MX missiles decision

ALTHOUGH urging support for the Strategic Arms Limitation Treaty recently signed by President Carter and President Brezhnev in Vienna, the Arms Control Association has warned that the benefits of the treaty will be undermined by the administration's decision to proceed with research and testing of a new generation of mobile missiles, known as MX.

In a statement released last week in Washington, the board of directors of the association say that, despite the closing of US intelligence bases in Iran, it considers the SALT II treaty to be adequately verifiable — a central issue of debate in the US Senate — and urges its ratification without substantive change.

However the ACA also expresses concern at the implications of the administration's recently announced plans to proceed with the design, development and deployment of the new MX missiles — possibly housed in and launched from underground trenches or

from holes on a "shell-game" principle — as a response to the increasing accuracy of Soviet weapons.

Criticising the lack of controls over increasing accuracy of ICBMs the ACA also says that "Deployment of more land-based, silo-destroying missiles will threaten nuclear stability and erode the basis of SALT still further if they are emplaced in mobile basing modes which multiply potential targets, forcing an adversary to programme additional warheads for targets which, in fact, contain no missiles."

The statement says that the problem of silo vulnerability is "largely hypothetical from an operational standpoint", and argues that deployment of the MX and similar systems by the USSR "will pose a far greater danger to American security, and to the SALT process and its accomplishments, than does the current prospect of a hypothetical Soviet attack on American land-based missiles." □

Rickets under control again in UK

VITAMIN D deficiency rickets is not a disease of epidemic proportion in Britain's Asian community. Dr John Ablett, a senior medical officer in the Department of Health and Social Security (DHSS), said last week there was now enough evidence to confirm that rickets is again on the decline in the UK. The DHSS now takes the view that rickets can be eradicated completely by the use of direct vitamin D supplements to children at risk. A policy of fortifying specific target foods — such as chapati flour — is therefore not necessary.

Rickets was first documented as a serious problem in the Asian community of Glasgow 18 years ago, and since then it has been observed in most of the larger Asian communities in the UK. Despite claims to the contrary by some doctors, the DHSS says the problem can be controlled by the traditional method of vitamin D supplements backed up by a health education programme — directed at the Asian community — about rickets and its adult equivalent, osteomalacia.

The claim that rickets is on the decline is based on figures from hospital admission records. Rickets is not a notifiable disease and data collection is not easy. However, the DHSS has used figures from its Hospital Inpatient Enquiry programme — in which 10% of all hospital admissions are analysed — from over 90 areas. The declining national trend, says the DHSS, is also supported by a survey involving general practitioners in areas with a large immigrant population.

The DHSS says there is evidence that Asian immigrants are entering the country with rickets or osteomalacia, which are quite common in India, Pakistan and Uganda. This means it should now be possible to identify the population most at risk. According to Ablett, the high risk are the infant children of recent immigrants who may be vegetarians, who come from a poor rural background, and who may have a family history of rickets and osteomalacia.

The DHSS's decision that it does not want a food fortification programme is in line with the recommendation of the department's Committee on Medical Aspects of Food Policy (COMA), which has studied the problem for three years.

The composition of milk cannot be altered without renegotiations of a European Economic Community directive (see *Nature* 270, 289; 1977). Margarine has a high composition of fatty acids (46% in some brands; butter has only 10% trans fatty acids) and is causing concern to DHSS officials anyway, as these acids have been implicated in some forms of cancer.

Chapati flour, the most serious conten-



One of the healthy ones: a hospital survey showed decline in rickets in immigrant areas

der, also has problems, largely over what concentration of vitamin D to use. A concentration sufficient to prevent rickets in young children would be dangerously high for adults consuming more flour per head. This is too risky, the DHSS argues, as there is now evidence to suggest that high vitamin D intakes result in high blood calcium and may cause cardiovascular complaints and even heart attacks.

The other problem with vitamin D in chapati flour is that the vitamin is unstable, and up to 50% may be broken down in cooking processes. These technical problems with the flour have still not been resolved, and millers are reluctant to embark on a vitamin D fortification programme unless forced to do so by law.

Finally, there is the problem of hypercalcaemia. The DHSS believes — and the COMA report due to be published next year will say this — that there is now good evidence to show that high vitamin D intakes in the 1950s caused the deaths of over 200 infants reported to have hypercalcaemia. Although the DHSS acknowledges that chapati flour fortified with vitamin D would not affect this age group, it says that high vitamin D intakes could cause problems for older teenagers and young adults.

In reaching its conclusion to rely on vitamin D supplements, COMA will point out that cod liver oil supplements reduced the incidence of rickets in children, from 13% in 1943 to almost nil by the end of the Second World War in 1945. If vitamin D supplements could eradicate the problem then, the DHSS argues, that it could do the same today.

However, there may be problems on the way. Preliminary evidence from Glasgow and Birmingham suggests that some 10% of children receiving vitamin D supplements are still developing rickets. These groups will require closer scrutiny to identify why this is happening.

Alastair Hay

UK scientists wait anxiously for £7.5m share-out

SCIENTISTS throughout Britain will learn in the next two or three weeks if they have earned a share in the Science Research Council's £7.5 million special investment in new equipment for university and polytechnic research departments. The cash distribution, which will benefit several hundred projects, follows the last government's improvements in the science budget and although the present administration has since reduced the level of increase, the SRC is committed to the move.

Originally only about £3 million had been considered for the equipment programme but the SRC has since been inundated with responses from scientists following its March appeal for applications for new research hardware. Now an expected £5.25 million alone is to go to chemistry, physics, biology and other departments covered by the SRC's science board and the remaining £2.25 million will be split between departments covered by the engineering, astronomy, and nuclear physics boards.

The decision to invest its share of the first £10 million increment in the science vote on equipment follows the SRC's prophetically accurate view that future increases were by no means guaranteed. So it was decided to spend the money as quickly as possible and grants for projects which would involve commitments to staff for several years were ruled out.

"We consider the best way that we can help universities and polytechnics to benefit from the present circumstances is to encourage them to pay particular attention to their needs for equipment", stated the March letter from the SRC to researchers.

This decision also recognised that many departments are facing severe financial problems in purchasing modern, sophisticated — and expensive — replacements for outdated instruments to provide the traditional "well-founded" university laboratory. This latter role is the function of the University Grants Committee, of course, but there are many intermediate areas between the domains of the SRC and the UGC.

Although the SRC stresses that it is not taking over any of the UGC's functioning, it is hard not to view the move as some form of help for the now financially-burdened UGC. However, in providing this new equipment, which will include items such as nuclear magnetic resonance instruments and mini-computers, the SRC is selecting those laboratories where it feels investment is best suited for its future research programme.

And the level of this need for new equipment can be judged by one estimate of £35 million for the total value of the applications received by the SRC. However

these applications were dealt with by the usual grant selection committees of the SRC and only those graded "alpha" were considered for equipment grants. Of these, about 50 per cent were selected for awards.

Now researchers are about to learn if their projects have been judged suitable.

Robin McKie

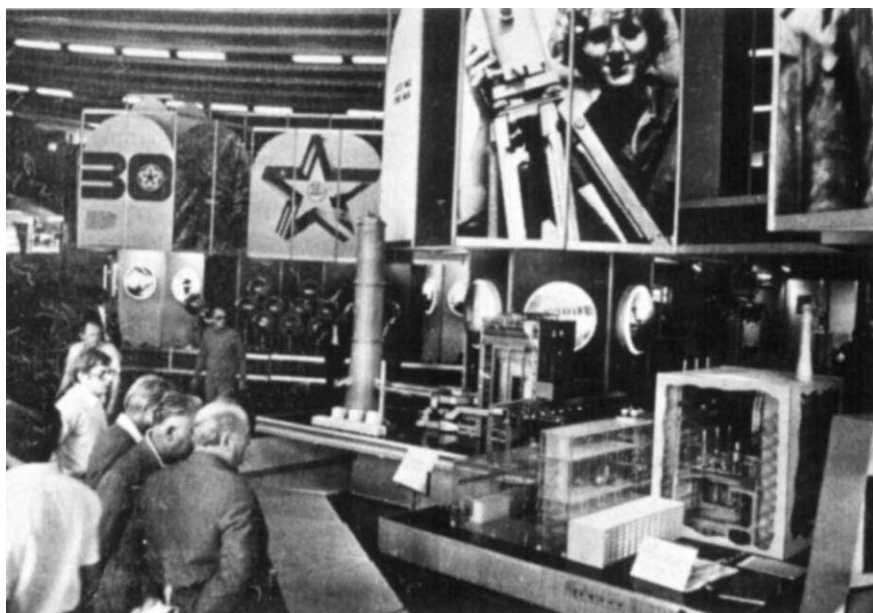
Investors refuse to back genetic engineering

ALTHOUGH two major British firms are investing in the industrial application of genetic manipulation techniques, some academics feel the UK is not putting enough money into transferring their research work into industry. The UK has not managed to adopt the US practice of using venture capital to set up small groups of academics and industrialists who develop research to the point where it can be applied to industrial processes. "Investment in the UK is very low", according to Professor K. Murray of Edinburgh University. "Most of the use of genetic manipulation is by small US venture capital groups."

At a meeting on "new horizons in industrial microbiology" at the Royal Society last week, several industrialists responded to these criticisms by saying that venture capital was available but that "research has to pay at some time". The economic climate in the UK meant that many firms did not think it was worth taking the risk of backing a new and uncertain technology. Even if the manufacture of drugs and vaccines by genetic manipulation and, more generally, the production of alternative fuels from organic material by microbiological methods, did become technically feasible, it would be some time before the new processes were economically competitive with current technologies.

A lack of communication between academics and industry was partly to blame for the failure to transfer new ideas. Academics tended to approach industry before their research was sufficiently developed for industry to take advantage of it. Industry, on the other hand, was not willing to take up a promising piece of research where the academics had left off. The result was a gap where good research could fall by the wayside. Dr J D Coombes of Hoechst, UK thought that matters might be improved if academics did not always have to approach the National Research Development Corporation first with their ideas and if they could hold patents.

For the scientists, a major worry was that government spending cuts might affect research. "Staff levels are running down and capital expenditure is down. We will not be able to conserve the strength we already have," said Professor B S Hartley of Imperial College. □



Poznan Fair: only the Poles had their own trading agency

Selling Polish science

THIS year, for the first time, the Polish Ministry of Science, Higher Education and Technology is appearing as an exhibitor in its own right at the Poznan International Fair, under the trading name Posteor; previously only projects developed in universities and polytechnics under its aegis have been on display.

Posteor is a financially autonomous organ of the Ministry of Science, somewhat analogous to the UK National Research Development Corporation. It was set up in 1973, a time when the Academy of Sciences first scrutinised the problems involved in the relationship between research and production. These discussions led to Poland's scheme of graduated 'problems' vital to the economy which are to be solved by science (23 November 1978 page 313); while, on the administrative side, Posteor was established to handle the implementation of new discoveries and technologies into production.

Implementation is a major problem throughout the Comecon bloc, but only Poland has adopted the idea of a special trading company. Indeed, by working through an existing foreign trading enterprise, Polservice, Posteor can earn valuable hard currency marketing know-how and licences abroad as well as protecting Polish patent rights. Through its exhibition at the Fair, Posteor hopes not only to increase its foreign outlets, but also to sell its technology and expertise at an earlier stage of development. "We can exhibit ideas, pilot technologies and know-how," explained one representative, "even if we haven't ironed out all the snags of full-scale production."

The small selection of projects on display is indeed wide-ranging: it includes a method for producing phosphates as a by-

product of sulphuric acid manufacture, and practical expertise in geodesy and water surveying, as well as an analogue system for measuring the field distribution near high voltage power lines — Poland will have its first 350 MW lines next year, as part of the Comecon supergrid. A method of cutting the petrol consumption of the average automobile by 15% underlines the importance of energy saving in the Posteor exhibits: other examples range from a cold-hardening process for worm-drive castings down to a method of freeze-drying whole potatoes. Indeed, energy conservation is a major theme of the entire Polish display, from the generating sets exhibited by Elektrim to the coal-mining pavilion with its placards extolling the leading role of the coal industry.

Following a winter when exceptional weather conditions led to a total halt in production, Poland is now rethinking one aspect of her energy policy, the proposed compensation deal with Austria. Under this agreement, Austria would build hydro-electric generating stations on the Bug, and in return would take two thirds of the current produced for the next 25 years. This scheme — like the proposal for a trans-Czechoslovakia pipeline to carry coal dust to Austria — is dear to the heart of Austrian trade minister Josef Staribucker, and such deals are normally popular with the Polish planners because they save hard currency by paying in kind rather than cash. But it is now most unlikely to go through, for, as the Polish vice-minister of trade, Edwin Wisniewski, told journalists at the Fair: "Coal and energy are now far too precious for compensation deals. We shall always be willing to sell them, so long as we have them to sell — but it must be strictly for cash." **Vera Rich**

news in brief

UK government scientists to strike: The Institution of Professional Civil Servants is continuing its industrial action through a series of selective strikes. Now scheduled for action are the government laboratories of the Agricultural Research Council and the Science Research Council. Also included will be the Central Veterinary laboratory at Weybridge, the Royal Aircraft Establishment in Farnborough and forensic scientists in the Home Office. The union is demanding pay rises linked to administrative grades for this year and an appropriate linking to outside salary structures for next year. (See leader comment.)

US-Europe to collaborate on testing cloning risks: The first results of a European attempt to assess some of the proposed hazards of the techniques of recombinant DNA (see p 811) help to define the way in which the next step in risk assessment should be carried out. It is a step, however, which cannot be carried out in Europe — because of the lack of animal facilities that meet the level of safety that is now required to avoid the conjectured risks that the experiment would attempt to assess. To escape from that predicament the European team will go to the US to collaborate with Dr W Rowe and his colleagues who have been carrying out a parallel study using the animal facilities at Fort Detrick. Both groups are testing whether the cloned genome of a tumour virus retains the ability to be infectious and to cause tumours. The Americans have already carried out some animal tests and will now be repeating them with the virus cloned in Europe.

SIPRI criticises increased NATO military spending: The Stockholm International Peace Research Institute, in its annual report, is sharply critical of NATO countries decision to increase their military spending by 3% a year. The SIPRI report states that the increased expenditure has been based on "dubious" propositions about USSR spending produced by US intelligence agencies. US intelligence has estimated that Soviet spending exceeds US spending, that it is taking an increased share of Soviet GNP and that it has been rising by 3% a year in real terms for a long time. According to SIPRI these estimates are constructed by converting labour intensive Soviet manufacturing into US capital intensive costs thus leading to an overestimate of real spending. "Valuing the military output of a much more labour intensive country such as the USSR at US prices distorts the actual situation" the report says. SIPRI also criticises the USSR policy of concealing defence spending, which it says contributes to the construction of exaggerated estimates of its spending.

The SIPRI report also warns that improvements in missile accuracy are leading to new nuclear war strategies. The old 1950s policy of mutual deterrence is giving way to a "counter-force" strategy. A new generation of missiles will be able to strike within tens of metres of hardened missile sites thus giving governments the "misplaced confidence that they can actually fight and win nuclear wars rather than simply deter them". (World Armament and Disarmament. SIPRI Yearbook 1979. Taylor and Francis Ltd, London.)

Sussex students win reinstatement: Sussex students Richard Flint and Shaun Fensom (21 June) were reinstated last Monday by a specially convened disciplinary committee. They had been "excluded" from campus for participating in a student union mandated examination disruption. The students were warned they would be excluded if they participated in further examination disruptions. Students have called off plans to disrupt conferences at the university this summer and the outstanding issues will be joined again in the Autumn. First year science students will decide at a mass meeting whether to continue the boycott of the preliminary science examination and the student rent strikers await the outcome of a university Senate meeting on student debt. The disciplinary committee decision followed another week of militant student action.

US prepares for military intervention in oil crisis: The US government announced Friday that it has established a "unilateral corps" of 110,000 men from Army, Navy, Marine and Tactical Air Force units. The corps will operate outside of NATO control and are ready for combat "in all theatres of operations where US interests are threatened". The special force will be dispersed among conventional military bases but will be mobilisable for rapid intervention at "hot points". Department of Defense head, Harold Brown said that the DOD currently is improving its sea and air transport facilities "so that we can get special units to various places distant from the US and Europe rapidly". Speaking at a press conference attended by Chief of Staff B. W. Rogers, Presidential adviser Z. Brzezinski, and Secretary of State C. Vance, Brown acknowledged that US dependence on oil imports was a serious potential "security" problem. The US gets 25% of its oil from the middle east. Brown emphasised that the US was seeking political solutions in the middle east and was consulting with moderate Arab states "which are understandably concerned about the possibility of outside intervention".

Strict new rules on the transport of nuclear waste: The US Nuclear Regulatory Commission recently published a strict set of rules covering the conditions under which spent nuclear fuel can be transported by road. These include the requirement that the NRC inspect and approve the route that each shipment is expected to take, that law enforcement agencies be informed in advance, and that each shipment be accompanied by guards knowledgeable about both the route, the cargo and emergency procedures.

The new rules have a dual purpose: to protect the public against possible accidents (all routes must, wherever possible, avoid major cities and urban areas), and to guard against the dangers of hijacking. To cover the latter, for example, the rules require that all vehicles be equipped with features that "permit immobilisation"; among proposals previously discussed for achieving this are dashboard buttons that would simultaneously blow out all the truck tires, explode the engine, and set off a wailing siren.

Antinuclear groups have already attacked the adequacy of the new regulations, which although they go into effect immediately, will be open for public comment for 45 days. They have challenged the first route that the NRC is now studying for approval between Norfolk, Virginia, and the Department of Energy's reprocessing and storage plant at Savannah River in South Carolina. This route would be used to transport spent fuels being brought in from abroad; anti-nuclear groups such as the Potomac Alliance argue that incoming ships could dock closer to Savannah River, and are therefore questioning the proposed route through Norfolk.

Bellerive Group attacks pro-nuclear lobby: The Bellerive Group, an international body of experts which includes Victor Weisskopf, a former director of CERN, has issued a stinging critique of conventional arguments in favour of nuclear power. Proponents of the orthodox pro-nuclear case have refused to answer the severe criticisms of their basic assumptions, the report says. These include assumptions about future electricity demand, patterns of energy use, and the possibility of expensive energy savings measures. Other criticisms are the inapplicability of nuclear generated electricity for transportation needs and the wastefulness of using nuclear generated electricity for space heating. The report also raises the dangers of nuclear proliferation and the possible dangers to civil liberties. The report warns that disaffection with society because of the nuclear issue "is greater than the nuclear industry and some governments that support it now realise. No free society can be governed without both trust and consent. Once trust evaporates government can be carried on only by increasing coercion".

Urban deprivation in the heart of the Amazon

by David Bousfield

THE city of Manaus lies high on the Amazon, at the junction of the Rio Negro and the Rio Solimões. During the last century it was the centre of the rubber industry in Brazil, and the magnificent Opera House, decorated largely with materials imported from Europe, still testifies to its former wealth and importance. Since those times, however, the area has been in decline and today the State Governor complains that the development grant intended for the whole of the Amazonas region is less than that given to the Tourist Department at Sao Paulo in the south. Recently Manaus was made a free port in an attempt to stimulate the local economy, but despite its attraction to Brazilian tourists, who travel thousands of miles to buy cheap cameras and hi-fi, this isolated town in the heart of the Amazonian forest retains all the problems of urban underdevelopment found elsewhere in the north and north-east of Brazil.

Manaus is fortunate, however, in having on its outskirts the principal administration and research complex of the National Institute for Amazonian Studies (INPA). This department has 107 scientific staff, while the other main department, at the Museu Goeldi in Belém has 51 scientists; in addition there are a number of research stations scattered over the Amazonian area. Under the Directorship of Dr Warwick Kerr, a geneticist from Sao Paulo, INPA's research projects have been directed at local problems. "INPA is always canalising effort towards the benefit of the people of Amazonia", he comments. Consequently research carried out by INPA includes work on fish farming, ecologically appropriate approaches to agriculture, malnutrition, and the impact of disease on settlement along the Transamazonian highway.

The rapid development of the fragile forest environment has created many problems for urban and rural colonists alike. For example, the 3,000 km Transamazonian highway, completed in 1975, was originally intended to allow families from the drought plagued north-east to colonize the forests. Yet the agricultural advice given to these would-be farmers by David Bousfield, of the University of Sussex, held a Nature Travelling Fellowship



INCRA, the Government colonisation agency, was not based on detailed research or planning. Indeed the route taken by the highway had already been decided, and some of the first colonists settled before the main soil survey work was carried out. Upland rice was envisaged as the principal cash and subsistence crop, but INPA geographer Nigel Smith has found that a better choice would be a more diversified crop base including manioc. Diversity reduces pest damage and means that income is not dependent on the success of a single crop. Indeed Dr Smith finds that the optimal farming strategies emerging from his work bear a striking resemblance to the methods of the Indians who used to live on the land now occupied by colonist settlements.

Mixing European and Indian crops

Since the colonists have little knowledge of which crops they should plant, additional work by INPA includes the selection of European varieties of fruit and vegetables such as carrots, cauliflowers and citrus, which are suitable for local soil conditions and climate. Attempts are also being made to reintroduce local fruit such as assái and bacabá into the Amazonian diet, and the Indian heritage has also been useful here since many Indian tribes actively cultivated the plants which produced the best fruit. The sapota, for instance, normally produces fruit weighing 80-100g, but the Tipuna Indians have managed to increase this to hundreds of grammes, and have trees which can yield up to 11 tonnes of fruit in one harvest.

Perhaps the most interesting example of INPA's concern for the Amazonian people, however, can be found in the nearby 'favela' in the Coroado district of Manaus. This slum town is built on private land invaded by the poor some six years ago. There are now over 25,000 people living in shacks made from wood, corrugated iron and even cardboard without any form of sanitation or a proper water supply. In 1977 INPA bought one of these houses and set up a school, thus providing a valuable base for research, and for establishing some interaction between the various departments of INPA and the people of the favela. The school's name, 'Escolinha da Abelinha', which can be translated as the 'Little School of the Busy

Bee', reflects the determination and sense of humour of Dr Kerr and its first teacher and director, INPA educationalist Margie Charlwood.

By concentrating on pre-school education (children aged between 4 and 6), INPA has made a considerable contribution to the nutritional and educational development of the children of Coroado. Some 48% of the children entering State schools at 7 have to repeat their first year courses, and as a consequence many become frustrated and never go back. Pre-school education helps to avoid this early trauma, and it is a strategy now advocated by UNICEF. Even the simple measure of providing regular meals improves exam success: 73% of children in the pre-school age range suffer from malnutrition and many show signs of physical and mental retardation. By isolating the main dietary deficiencies and by trying to counteract them at the school, Charlwood and INPA nutritionist Roger Shrimpton hope to improve infant feeding habits and nutritional status.

Traditionally much of the food for Manaus has been imported. In 1975, for example, the region only produced 25% of its requirement of rice and meat, only 12% of its milk, and it imported nearly all its fruit and vegetables. The resultant expense creates some rather unexpected dietary deficiencies. Despite being surrounded by a forest containing many edible species of plant, the inhabitants of the city consume per capita only one-half the amount of fruit and one-third of the green vegetables eaten in Sao Paulo, and so deficiencies of vitamin A, thiamin, riboflavin and iron are common.

Bad diet preferences are also partly to blame for nutritional problems. In the days of the rubber barons the best pâté and champagne were readily available in Manaus, and today the freedom from import tariffs continues to encourage specialist tastes. The current school meals programme run in State schools and backed by the FAO includes Norwegian cod, Dutch cheese and crystallised fruit to supplement children's diets. INPA workers argue that this programme is merely creating markets for richer countries' unwanted products rather than teaching children how to feed themselves. Consequently attention is now being fo-



Incongruous Manaus: amid the poverty, the Opera House is a reminder of former glories

cused on the possibilities of using local varieties of fruit and vegetables.

Various diets are being introduced to the children of the Bee School and their acceptability and nutritional impact assessed. The mothers are actively associated with the running of the school and contribute to the preparation of these meals, ensuring that the educational process is not restricted to the children and the bond between the school and its community is strengthened. At the same time simple rules of basic hygiene and nutrition are being incorporated in the curriculum. An elementary reading and spelling exercise book, or 'cartilha', written by a team including Dr Kerr contains advice on which foods contain calcium and how to take care of teeth.

It is too soon to assess the project's eventual success, although the number of children attending the school has risen from 30 to 220 during its first two years, and after six weeks of the supplementary school meals their average weight increased by two kilogrammes. Once a cheap and nutritious diet has been developed, INPA would like to see the scheme extended to other 'favelas' in the area and eventually become integrated as part of a scheme of development for the whole region.

Unfortunately the relationship between science and the solution of social problems elsewhere in Brazil is much less apparent than in Manaus. Many scientists I spoke to in the south felt that the social problems of the country (one-third of Brazil's children, some 16 million, live in slum communities) could be solved simply by political reform and government aid. But it was also clear that in the sophisticated intellectual climates of cities such as Brasilia and Rio de Janeiro a preoccupation with Western science is leaving little time for considering how the problems facing the urban and rural poor might be approached. Now Kerr and his co-workers have lead the way, will others follow? □

Asian scientists agree on development issues

Yonguth Yuthavong of the Department of Biochemistry, Mahidol University, Bangkok reports on a lively meeting in Kuala Lumpur

SCIENTISTS from 21 South and South-East Asian countries met last month in Kuala Lumpur, Malaysia, and agreed on six basic principles to improve science and technology in their countries.

The meeting, organised by the Committee on Science and Technology in Developing Countries (COSTED, a body set up by the International Council of Scientific Unions), concluded that:

- Since the general level of indigenous scientific and technological capability is very low, and varies greatly among developing countries, more emphasis should be placed on regional sharing of scientific competence.

- There should be increased representation of scientists and technologists from developing countries in international bodies dealing with science, technology and development. Possible measures include travelling fellowships and the establishment of international institutions in the developing world.

- Effective and socially relevant science and technical education is one of the most important factors contributing to development. International support is needed for preparation of suitable educational material and for training of personnel, but care should be taken to promote self reliance in the education programmes of the developing countries.

- Suitable scientific and technological information systems should be established, oriented towards important issues in development.

- To make UNCSTD a success, a follow-up mechanism to implement its decisions must be speedily established.

- It was of particular importance, the meeting decided, to define the goals of development as clearly as possible. The meeting emphasised the provision of basic needs for people — rather than high per capita GNP. The Association for the Application of Science to Human Affairs (ASHA), composed of a group of scientists in India and supported by COSTED, has proposed the ASHA Development Index (ADI) based on parameters of health, literacy and employment ($ADI = \text{per capita GNP} \times \text{growth rate} \times \text{employment} \times \text{literacy} \times \text{life expectancy fraction}$ [70 yr = 1.00]/birth rate $\times$ infant mortality). The ADI was found to vary from 43 for poor countries to over 10,000 for rich countries. The goal advocated by ASHA is an index of over 2000 by the year 2001. While there is at present a general correlation between GNP and ADI, the meeting agreed that strategies for future development should aim to raise ADI, rather than GNP simply through rapid industrialisation with depletion of resources and urbanisation.

The lack of an effective scientific and

technological infrastructure in the developing world is reflected by various indicators: number of scientists and technologists, number of institutions, amount and quality of publications and other output. A survey of papers published in international journals showed that a very small fraction originated from developing countries. It is striking that India contributes about 5,000 papers per year (in journals covered by *Current Contents*), half of the total output from all developing countries and far more than South Africa or Brazil. The amount of research output alone therefore does not bear a simple relationship with the state of development of a country. It was generally agreed that to promote development in a poor country most effectively, not only must the research be of high quality but also the deal primarily with unique resources or pressing problems of the country. Good examples are rubber research in Malaysia and diarrhoeal diseases research in Bangladesh. This demands keen awareness of the researchers on various aspects of priority research areas — an awareness which is lacking, given the present communication gap between the scientists, the industrialists and the general population in the developing world. A remedial mechanism was proposed in which the scientists, the industrialists and the citizens could mutually interact in forums organised by the professional societies and government or private agencies. In addition, novel structures of reward and recognition should be devised to encourage research interests in development-related areas.

The meeting also emphasized the value of science as creative activity for the enquiring mind. The scientist, however, tends to become alienated from his society if his work has little relevance to its problems. A conscious effort should therefore be made by the scientific community to bring social relevance into their work, while preserving the role of making enquiry for more basic knowledge at the same time. A proper balance between basic and applied science would depend on the level of development, resources and constraints of each developing country.

It was felt that there was a need for novel scientific and technological information services orientated towards development problems of the Third World. Suitable services should be available not only for scientists and technologists, but also for administrators, field or extension workers and the general population, mostly illiterate farmers and small scale village industrialists. The problems foreseen are enormous, and

proposed solutions ranged from creation of a new information system on science and technology for development, to provision of information materials to the developing countries at a moderate cost, and promotion of public understanding of science and technology through mass media. International information agencies, scientific unions and private publishers can play substantial roles in these schemes. It was argued, for example, that sale of books and journals to the developing countries is only a minor fraction of the total sale, and the publishers should consider reducing the prices to a level which these countries can afford. Schemes for book donation such as operated by UNESCO should also be made more effective.

Nowhere is the need for public understanding of science and technology more acutely felt in the developing world than in the fields of health and agriculture. Many tropical diseases are largely preventable: hookworms are prevented by wearing shoes, cholera by having clean water supply, xerophthalmia by eating food containing vitamin A, etc. The yields of major food crops can be

increased several fold through proper fertilizer supplies, pest management and irrigation. Apart from the fact that money and supplies are simply not available, the necessary information is also not accessible to the majority of the rural population. Technology transfer for village industries also present similar problems. The development of extension services and non-formal education deserve high priority.

The strengthening of the scientific and technological capacity of the developing world will be both expensive and time-consuming. Some delegates even expressed despair that this goal will ever be significantly achieved in the majority of developing countries. An alternative goal was proposed whereby a group of countries within the same region would share their resources and pool external aid in a limited number of large and effective institutions. While this arrangement could possibly be of benefit for certain projects which need a considerable amount of scientific infrastructure, its main drawback is the continuing dependence of the smaller and less developed countries, their big brothers merely being in the same

region rather than somewhere to the North. Whatever the strategy, it was clear that much more co-operation from the developed countries should be forthcoming. This will have to include provision of scientific equipment and maintenance facilities, information, help in scientific and technical education, exchange of scientists and technologists. The developing countries themselves will have to try harder to support their scientific and technological activities, including education.

Developing countries often have an asset in abundance of natural resources, solar energy supplies and a climate suited for rapid bioconversions. The meeting agreed that these local advantages should be utilized, which implies that the problems for science and technology will often be very different from those in developed countries. It was proposed that a consortium of experts from the developing countries be formed to deal with training and development of technology for optimum utilization natural resources. It is hoped that international agencies including COSTED will take up this matter further. □

Hunting Mongolian dinosaurs

COMECON, in the thirty years of its existence, has provided the framework for numerous scientific cooperation projects. One of the most interesting and most unusual was the Polish-Mongolian series of palaeontological expeditions of 1963-1971, organised by Dr. Zofia Kielan-Jaworowska of the Polish Academy's Institute of Palaeozoology. (For the latest results, see p 792.)

These expeditions not only produced a wealth of scientific material on dinosaurs, dinosaur eggs and mesozoic mammals, which is gradually being published in *Palaeontologia Polonica* (the final report is expected to extend to some 16-20 thick volumes; No. 8 is now about to appear); they also resulted in an excellent "popular" book on palaeontology — Dr. Kielan-Jaworowska's *Hunting for Dinosaurs*. Most important, perhaps, as I learned when I spoke with Dr. Kielan-Jaworowska in Warsaw, they provide a fascinating commentary on how formal "scientific cooperation" can work.

Not that the Gobi expeditions were entirely typical! Cooperation agreements within Comecon tend to take place between the Soviet Union and one or more of the smaller member-countries — a major project without Soviet participation is somewhat rare. In fact, Dr. Kielan-Jaworowska explained, at the beginning there was some talk of Soviet participation, but the Soviet Palaeontological Institute was "too busy".

The idea for such an expedition arose in 1961, when the representatives of the Comecon Academies of Science met in

Warsaw. During this meeting, a cooperation was signed between the Polish and Mongolian Academies, and Dr. Kielan-Jaworowska put forward a proposal for joint palaeontological expeditions. "This was my private dream," she explained, "and also the dream of many workers in this institute. Mongolia is an unusual country, especially for vertebrate palaeontology. The odd thing is that we were invertebrate palaeontologists at the time!"

In due course, Dr. Kielan-Jaworowska was appointed to lead the expeditions. One of the main practical problems was, of course, that Poland is among the most scientifically advanced Comecon countries, while Mongolia is relatively backward. For the project to be genuinely bilateral, some preliminary training was necessary. "We had to help the Mongolian Academy," she said. "We had to teach their scientists. They came during their training to our institute and we published some joint papers. At that time there were about ten Mongolian palaeontologists working in Ulan-Bator. Two of these — Demberelyin Dashzeveg (a specialist on mammals) and Rinchen Barsbold (dinosaurs) — were very gifted."

The field-work of the expeditions proved successful beyond all hopes. Major finds included dinosaur eggs, fossil tortoises, many new genera of dinosaurs, and, for the first time, well-preserved skulls of multituberculates, which threw new light on the mesozoic "dark ages" of mammalian history. Subsequent study of the multituberculate finds showed that these non-placental mammals were closely related to



On the trail: Dr Kielan-Jaworowska (right) and colleague

the monotremes as far as brain structure was concerned, thereby raising the further question of whether they were viviparous or oviparous.

Poland has an excellent network of museums, Mongolia has simply the municipal museum in Ulan Bator. One might be tempted, therefore, to imagine that the bulk of the material will remain in Poland. "Not at all," said Dr. Kielan-Jaworowska. "The agreement is that after we have studied the material, part of it will be sent back to Ulan Bator."

"There will be a true pay-off for both sides," she added. "We have got some most interesting material, which throws great light on some of the most important questions of evolution. And the Mongolians will get good material and the basis for a good museum."

Vera Rich

news and views

Guest species in minerals and other crystals

from J.M. Thomas

So widespread is the occurrence of isomorphous and other forms of substitution that their nature and consequences are the concern of chemists, solid-state physicists and mineralogists alike. When a guest species is present either in small quantities or in rather large but non-stoichiometric amounts, the determination of its precise crystallographic location is seldom a simple affair. A good deal of the evidence pointing to the phenomenon of site substitution came originally, and indirectly, from a combination of simple chemical principles and accurate wet chemical analyses. It was soon established that, among the numerous kinds of silicates that constitute the rock-forming minerals, there is a tendency for Al^{3+} ions to occupy tetrahedral sites normally tenanted by Si and for Mg^{2+} to replace Fe^{2+} or Al^{3+} in octahedral sites. Similarly Li^+ replaces Mg^{2+} in sites of sixfold coordination. The negative charge borne both by the framework zeolites, that nowadays play such a crucial role in the catalytic cracking and synthesis of hydrocarbons, and by the sheet silicates, which are responsible for the cation-exchange capacity of clays, is a direct consequence of this kind of substitution. Site-substitution and site-selection are also implicated in trace-metal segregation and enrichment; and thermodynamic arguments that apply to geothermometry and geobarometry (Navrotsky *Prog. solid state Chem.* **11**, 203; 1976) rely, in turn, on experimental methods of ascertaining site identity and occupancy.

X-ray crystallography, from its inception, has, in favourable circumstances, offered a more or less direct means of assessing the extent of site substitution. But in view of the factors that govern the scattering of X-rays, this approach is not well-suited to discriminate ions of elements possessing closely similar atomic numbers. Thus, whereas the pairs Ca^{2+} and Mg^{2+} , Sr^{2+} and Ba^{2+} , or K^+ and Na^+ are readily distinguishable both on grounds of scattering power and ionic radius, it often proves difficult to identify Si^{4+} sites that are occupied by guest Al^{3+} . X-ray methods encounter a similar difficulty when Fe^{2+} is replaced by Mn^{2+} or vice versa, especially if the extent of substitution is small or if the guest ions are randomly distributed within the host matrix.

In seeking alternative approaches, much progress has been achieved by spectroscopic methods, which involve monitoring the frequency, intensity and band profiles of absorptions in the infra-red and visible regions of the spectrum. Mössbauer spectroscopy, though of restricted applicability, has contributed much to knowledge of site population and preference, especially in ferrous and ferric ions in amphiboles, pyroxenes, garnets and biotites (Bancroft, Williams and Burns, *Am. Mineral.* **56**, 1617; 1971). (Conversion electron Mössbauer spectroscopy, which is even more restrictive in its applicability, has also been particularly powerful in ascertaining site characteristics in certain sub-surface phases — see Tricker, Winterbottom and Freeman *J. Chem. Soc., Dalton Trans.* 1289; 1976.) Recently, however, electron spectroscopy has burgeoned, and although its direct application has contributed little to the elucidation of site occupancy, a rather novel development (termed XPD for reasons given below) has now made possible new insights into the crystallography of various naturally occurring and chemically modified minerals. The technique is simple, straightforward and widely applicable, provided reasonably sized single-crystal specimens are available.

X-ray-induced photoelectron spectroscopy (XPS) entails the measurement of the kinetic energy of electrons liberated when a material is irradiated with monochromatic X-rays. From the known energy of the latter the binding energy of the core electron (for example, K 2s, Al 2p, Fe 3p) may be evaluated. The precise value of the core-electron binding energy is a function of the environment experienced by the atom from which the electron is emitted. But the magnitude of the 'chemical shift' in binding energy exhibited by a given ion in two or more crystallographically distinguishable sites is disappointingly small (Adams, Thomas and Bancroft *Earth Planet Sci. Lett.* **16**, 429; 1972) and inadequate to serve as a trustworthy basis for site identification. Fortunately a more subtle use of XPS can be envisaged. This entails monitoring the 'diffraction' of the X-ray-induced photoelectrons (XPD) as the take-off angle of the emitted electrons is varied. Although the existence of this diffraction has been

known for some years (Siegbahn, Gelius, Siegbahn and Olson *Phys. Lett.* **A32**, 221; 1970; Fadley and Bergstrom in *Electron Spectroscopy* (ed. Shirley) 233; North Holland, Amsterdam, 1971) its potential value in structural elucidation of the near-surface regions of solids does not seem to have been recognised previously, in spite of the early observation by Fadley and Bergstrom (1971) that the angular dependence of the XPS signal from silver dissolved in single-crystal gold was essentially identical with that of the gold. The structural elucidation of complex solids by XPD has been described recently by Adams, Evans and Thomas (*J. Am. chem. Soc.* **100**, 3260; 1978).

Suppose the angular variation of the intensity of the Si 2s photoemitted electrons of phlogopite mica is monitored. The intensity fluctuates because of the (multiple) diffraction that the electrons suffer between the point of generation inside the crystal and their escape into the vacuum. If, as was originally suggested by Mauguin (*C.r. hebd. Seanc. Acad. Sci.* **156**, 1246; 1928) and Pauling (*Proc. natn. Acad. Sci. U.S.A.* **16**, 123; 1930), Al^{3+} ions occupy some of the tetrahedral sites normally taken up by Si^{4+} , then the angular variation of Al 2p and Si 2p photoelectrons (or of Auger electrons emitted from these atoms) should be identical, a situation which would certainly not obtain if Al^{3+} were preferentially or additionally resident in octahedral sites. By taking intensity ratios (for example, Si 2p/Al 2p or F 1s/O 1s), rather than absolute values for single core levels, sources of experimental and related uncertainty (caused, for example, by eccentricity of the specimen mount), are eliminated, and comparison of site substitution in one specimen with near- or exactly-equivalent substitution in another is rendered possible. (Moreover, the essential invariance of K 2p/2s and Si 2p/2s ratios with angle demonstrates that differences in electron wavelength and radius of ionised shell are of secondary importance.) Using this simple qualitative approach, Evans and collaborators (*Phil. Trans. R. Soc.* in the press) have re-examined critically the hitherto accepted site assignment of a range of micas. In addition to presenting direct proof that Al^{3+} ions are indeed accommodated only in the (Si^{4+}) tetrahedral site in phlogopite, they also demonstrated that the Mg^{2+} ions

in vermiculite and the Al^{3+} ions in lepidolite are in identical crystallographic environments, both being situated at octahedral sites. Moreover, XPD shows that ions (such as Na^+) present in muscovite in quantities too small to be detected by conventional X-ray methods, occupy sites that are similar, but not exactly equivalent, to those occupied by the interlamellar K^+ ions which they replace.

By its nature the technique is more reliable the smaller the extent of substitution, and it does not require the guest species to be ordered (contrast X-ray diffraction). And although it is biased in favour of the sub-surface regions (about 100 Å) of the crystal — because of the magnitude of the electron escape depth — it possesses further advantages in that, combined with measured XPS intensities and known photoelectric cross-sections, it affords insights into the degree of hydration of exchangeable cations. Thus it has been established (Evans, Adams and Thomas *Phil. Trans. R. Soc.* in the press) that, whereas interlamellar Ca^{2+} or Pb^{2+} ions in weathered or solution treated vermiculite are shrouded with a hydration shell, interlamellar K^+ ions, on the other hand, are bare. In similar vein, Evans (in preparation) has recently shown that substitutional titanium ions in the mineral biotite occupy both tetrahedral and octahedral sites (the approximate percentage population being 30 and 70 respectively). Furthermore, he reported at the European Conference on Surface Science (ECOSS2) in Cambridge, March 1979, that, by recording intensity variation as a function of azimuthal angle (at a fixed polar angle) information about site symmetry (of the photoemitting atom) may be gleaned. In this respect, XPD is formally akin to the more strictly surface tools that utilise angular variation of core-level electrons liberated (by X-rays or synchrotron radiation) from adsorbed phases. These tools have been applied with conspicuous success by Kono, Fadley, Hall and Hussain (*Phys. Rev. Lett.* **41**, 117; 1978) who succeeded in extracting information on both the symmetry and bond lengths associated with oxygen chemisorption on copper, and by Woodruff, Norman, Holland, Smith, Farrall and Traum (*Phys. Rev. Lett.* **41**, 1130; 1978) who arrived at quantitative surface structural information relating to the adsorption of Te and Na on $\text{Ni}(100)$ faces.

It seems clear that, provided experimental methods can be evolved to cope with smaller single-crystal specimens, a wide range of structural and thermodynamic problems in geochemistry, as well as in materials and engineering science, should be clarified by the application of XPD. □

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Proline and folding proteins

from Robert Freedman

A FOUR-YEAR-OLD hypothesis that the imino acid proline has a special role in the folding up of proteins has been confirmed, refined and extended by several, almost simultaneous publications from laboratories in Britain, the United States, Switzerland and the Federal Republic of Germany. One of the papers gives a detailed picture of how the complete three-chain protein collagen, the main protein in tendons and cartilage, folds itself up. Proline is unusual among the components of proteins in that it is an imino acid rather than an amino acid. This gives it unique and long-recognised properties of restricting the folded structures a protein can take. But it has become apparent recently that prolines may also control the rate at which proteins can fold up from an unfolded state.

Thirty years ago, Linus Pauling showed that in most peptide bonds, the side chain groups of the amino acids (R_1 and R_2 in Fig. 1) are held as far apart as possible in a *trans* configuration for the peptide bond. This is much preferable to the more crowded *cis* arrangement. But for bonds involving the NH group of proline both *trans* and *cis* arrangements are possible; the *trans* configuration is still slightly preferable and is found more often in folded proteins, but in an unfolded protein the *cis* form of the bond could easily occur. If that were the case then the process of getting proline peptide bonds into the right configuration would be important in the folding up of proteins.

In 1975, Brandts, Halvorson and Brennan (*Biochemistry* **14**, 4953) collected data on the folding of proteins and pointed out two important facts. For many proteins the folding process occurs in two phases and the rate of the slow phase is very similar to the rate at which proline peptide bonds isomerise — interconvert between *cis* and *trans*. To explain this, they proposed that only the unfolded molecules with all their proline-containing peptide bonds in the correct configuration could fold up. So the fast phase corresponded to the folding of the molecules which were all correct at the start of folding, while the slow phase corresponded to the slow isomerisation of the remaining molecules.

Gradually the data have accumulated to support this interpretation. Over the years Baldwin and colleagues at Stanford have analysed the slow and fast phases of refolding of ribonuclease in great detail. Now, he and Schmid have shown that the slow phase is the result of slow isomerisation of peptide bonds involving proline, but their quantitative data do not exactly agree with the model originally put forward (*Proc. natn. Acad. Sci. U.S.A.* **75**, 4764; 1978). This proposed that all the peptide bonds in the unfolded protein would have to isomerise to the correct configuration before folding could begin. The data of the Stanford group suggest that not all the bonds need to be correct; there may be some 'permissive' proline residues which can be fitted in, either in the *cis* or the *trans* configuration.

Similar modifications of the theory have been suggested by Creighton, who has considered the problems facing a large protein containing a lot of proline residues (*J. molec. Biol.* **125**, 401; 1978). Whereas the slow phase of refolding for the small proteins mentioned above is of the order of a few seconds, Creighton shows that for a protein containing 20 proline residues it would be about 10 min, and would stretch into hours for a protein with 30 proline residues. These figures are so unreasonably large that modifications to the model have to be considered. There are two related possibilities. The protein could be divided into domains each of which can fold independently; the folding of each domain would be as fast as that for a small protein and the whole molecule would soon be fully folded. Alternatively, without a clear division into domains, a protein could nevertheless fold itself up bit by bit, the region around a 'correct' proline folding to its final conformation without having to wait for all the prolines in the molecule to be in the right configuration.

The former of these proposals, domain organisation, is probably what happens in most globular proteins. But for collagen, a fibrous structural protein, there is now direct evidence for the second — 'bit by bit' — hypothesis, from the work of Bächinger, Bruckner, Timpl and Engel (*Europ. J. Biochem.* **90**, 595, 605; 1978). This collaborative group based at Basle and Martinsried has isolated a fragment of procollagen which on a small scale resembles the whole collagen molecule. This fragment (peptide Col 1→3 of bovine type III procollagen) consists of three poly-

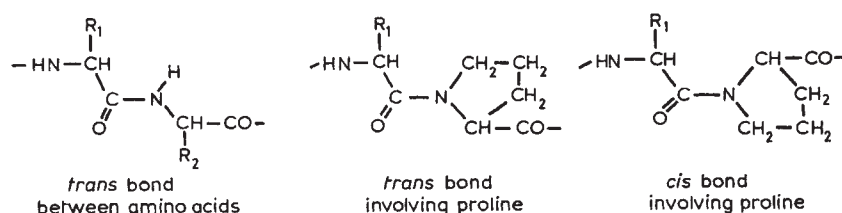


Fig. 1



Fig. 2

peptide chains, the central regions of which are twisted round each other in the 'triple-helical' structure characteristic of collagen. At the end of the triple-helical region, the chains are fixed to each other by a kind of knot formed by disulphide bonds between the chains (Fig. 2). Bächinger *et al.* have unfolded this fragment and studied its folding as a model of the folding of collagen itself.

The interesting point here is that the Col 1-3 fragment, like collagen itself, is very rich in proline residues and hydroxyproline residues. The whole fragment has 27 such residues in each of its three chains so that they make up 20 % of the protein; in the triple-helical region they comprise one-third of all the residues. But Col 1-3 folds into its complex triple-helical structure in not much more than a minute. Is this speed consistent with the hypothesis that proline peptide configuration is crucial to folding?

The group from Martinsried and Basle showed that peptide bond isomerisation was important by a neat experiment. When they refolded Col 1-3 from the unfolded state it folded in two phases. But the proportion which refolded fast was greatest when they used material which had only just been unfolded. In other words, if you unfold the protein and immediately try to refold it, most of it can refold rapidly;

but if you wait before refolding it, the proportion which refolds rapidly, diminishes progressively. This suggests that immediately after unfolding all the bonds are in the correct configuration for folding, but that they gradually isomerise and incorrect configurations accumulate. All the thermodynamic parameters of the folding and unfolding also showed that the isomerisation of proline peptide bonds was the rate-determining step.

So how are all the proline peptide bonds isomerised so rapidly? The answer is that the process can occur bit by bit. The disulphide knot is a nucleus from which folding can begin. Then the three chains can begin to twist themselves up correctly until they come to a proline in the wrong configuration. Once that has isomerised they can proceed as far as the next wrong one and hence the folded structure is rapidly propagated. Because the protein here is essentially a linear cable, rather than a tangled mass like globular protein, folding can proceed in this stepwise manner, rather than being an all-or-none process which depends on all the proline residues being in the correct configuration at the start. □

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West African membrane controversy

from Peter J. Smith

SOME years ago Turcotte and Oxburgh (*Nature* **244**, 337; 1973) introduced the fascinating concept of 'membrane tectonics' to explain certain aspects of the internal tectonic behaviour of the Earth's lithospheric plates. The idea is simply that plates, being thin shells in relation to the Earth's radius, should act as membranes, the theory of which has long been familiar to civil engineers and the like. Specifically, as the Earth is an oblate spheroid rather than a perfect sphere, any plate moving from one latitude to another will experience a change in curvature and all that results from it. Generally a plate travelling away from the equator will find its curvature decreasing and its periphery under tension, whereas a plate approaching the equator will increase its curvature and experience peripheral compression. A moving plate will thus be subjected to deformation, stresses and consequent secondary phenomena such as fracturing.

As possible examples of membrane tectonics in action Turcotte and Oxburgh suggested Hawaii and East Africa; but later Freeth (*Earth Planet. Sci. Lett.* **38**, 298; 1978) was to put forward what he held to be "an even better example", namely, West Africa and the Gulf of Guinea. It is difficult of course to apply simple membrane theory to a plate containing an inhomogeneous continent with an irregular outline, not least one that for more than 100 M yr has been growing (by accretion at the mid-Atlantic ridge) and parting from a once-joined continent (South America). Nevertheless, Freeth managed to convince himself that in the West African rift zone (chiefly the Benue, Yola and Gongola rifts), compression and tension occurred in the way one would expect for a region first approaching the equator and then receding from it in the northerly direction.

Thus as the West African rift system

moved towards the equator from the south 90-40 M yr ago it underwent compression, resulting in folding, the eruption of andesites (implying subduction) and the development of mylonite zones. But while the region was in the vicinity of the equator about 40 M yr ago there was little or no tectonic activity. Then during the past 30 M yr or so, as the area has moved away from the equator, there have been new rifting and volcanic activity, most notably those associated with the development of the so-called Cameroun volcanic line extending from the offshore islands in the Gulf of Guinea into central Cameroun.

Freeth's presentation appeared fairly convincing in a hand-waving sort of way, or at least it was something worth thinking about. But it failed to convince Thorpe and Wright (*Earth Planet. Sci. Lett.* **42**, 327; 1979) who have now objected to it on two main grounds. They point out first that during part of the crucial period up to 40 M yr ago, when the Benue rift was supposedly under membrane compression, the region was actually under net tension — an unexplained inconsistency acknowledged by evidence quoted by Freeth himself. Moreover, during the past 40 M yr, while the region was supposedly under membrane tension as a result of drift away from the equator, it may not have been moving northwards at all. The palaeomagnetic evidence appears to be more consistent with an Africa more or less stationary during this period, an interpretation that opens the way to the alternative hypothesis that "volcanism and tectonic activity are consequences of the focussing of sub-lithospheric thermal anomalies onto a slowly-moving or stationary plate".

The second point made by Thorpe and Wright is that membrane theory postulates peripheral or marginal compressions and tensions in moving plates, a geographical factor that Freeth first acknowledged but subsequently appeared to ignore. The Gulf of Guinea may have been plate-marginal when Africa and South America first separated, but ever since then it has in effect been migrating further into the African plate as the South Atlantic has grown. Nor is the volcanic activity associated with the mid-plate setting of the Cameroun volcanic line unusual in the context of the African continent as a whole; such activity occurs throughout the non-cratonic areas of North Africa and is not even preferentially distributed towards plate-peripheral zones.

A third, rather less central, point made by Thorpe and Wright is that "there is no evidence for any sea-floor spreading and subduction in the Benue trough". This, as students of the region will know, is a highly contentious point. In his response Freeth (*Earth Planet. Sci. Lett.* **42**, 329; 1979)

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devotes considerable attention to it; and if the power of detailed referencing and a sense of conviction were the sole criteria, he would win hands down. It is a pity, though, that in following up this issue Freeth fails to do justice to the two more crucial criticisms. He makes no mention at all of the question of the marginality of compression/tension. Nor does he pursue the problem of compression or tension prior to 40 M yr ago. On the other hand, he does deal with the continental drift issue, pointing out quite rightly that the palaeo-continental maps he used, namely those of Smith (A.G.) and Briden *Mesozoic and Cenozoic Palaeocontinental Maps*, Cambridge University Press, 1977) incorporate the most comprehensive data set available.

So where does that leave West African membrane tectonics? In a highly unsatisfactory state, unfortunately. As far as the impartial observer is concerned, a reading of the current literature on the controversy suggests that the opposing cases are equally good, or rather equally bad. In the credibility stakes Thorpe and Wright have the edge; but that may be because by the very nature of things it is easier to put a spanner in the works than build a nice bit of machinery. The moral is that it would probably be better to look to a less complex area if the aim is to assess the reality or otherwise of membrane tectonics. If the object is to explain the evolution of the West African rift system, on the other hand, someone is going to have to put forward some rather more convincing evidence one way or the other. □

A diet of worms

from J.E. Sulston and J. Hodgkin

FIVE years ago Brenner published an extensive genetic characterisation of the small free-living nematode *Caenorhabditis elegans*. Largely as a result of his pioneering work, this organism has become the subject of many different lines of research. Last May more than 120 researchers met at Cold Spring Harbor to discuss recent findings in *C. elegans* biology*.

This animal is a self-fertilising hermaphrodite whose rapid life cycle (3.5 days) facilitates genetic manipulation. Its small size (1,000 $\mu\text{m} \times 60 \mu\text{m}$ at maturity) and transparency facilitate the observation of cells *in vivo* and also make possible the use of whole mounts for histology. The subsequent location of defined regions and serial sectioning for electron microscopy are now routine. Development is predominantly mosaic, and because of the small and invariant number of cells (550 at

hatching), the behaviour of cells in the wild type can be defined exactly. The genome is small (haploid DNA content twenty times that of *E. coli*) and estimates of the number of essential genes are low (2,000–4,000).

Perhaps the most important advances reported at the meeting were in the isolation of cloned DNA fragments, in the refinement of genetic methods, and in the characterisation of developmental mutants. With these tools, progress towards analysis of the genes controlling development seems feasible.

S. Emmons (University of Colorado, Boulder) described work on randomly cloned restriction fragments: detectable differences between the Bristol and Bergerac races of *C. elegans* could be demonstrated for 15 % of these fragments, and such fragments can therefore be assigned to particular regions of the genetic map. No differences between germ line and somatic DNA were detected for 50 different fragments although such differences (chromatin diminution) are known to occur in certain other nematodes. Analyses of ribosomal DNA (J. Files, Boulder) and of three tRNA genes (T. Tranquilla, MRC Laboratory of Molecular Biology Cambridge, UK) were reported; the repeat length of the ribosomal DNA is short (6,800 base pairs).

Muscle assembly can be studied both biochemically and genetically in *C. elegans*. A MacLeod (Cambridge, UK) has cloned a cDNA fragment corresponding to the major myosin heavy chain species. The structural gene for this protein, *unc-54*, has been subjected to detailed genetic analysis by R. Waterston (Washington University School of Medicine, St Louis), who presented a fine structure map, and by P. Anderson (Cambridge, UK), who has obtained more than 100 new *unc-54* mutations by a selective technique. Some of these mutations are small deficiencies that allow mapping of the genes adjacent to *unc-54*.

At least 17 other genes affecting the body wall muscle structure are now known, which map throughout the genome (J. Zengel, Baylor College of Medicine, Houston; Waterston, St Louis). Fine structure maps of two of these genes, *unc-15* and *unc-22* were presented by A. Rose and D. Moerman (Simon Fraser University, Burnaby): *unc-15* is the structural gene for paramyosin, but the product of *unc-22* is unknown. It may interact with the *unc-54* (myosin) product since some *unc-54* mutants suppress some *unc-22* mutants. This suppression appears to be specific. On the other hand, two pleiotropic suppressors, *sup-5* and *sup-7*, have been identified that suppress mutations in a wide variety of genes (Waterston and others). Only null alleles appear to be suppressed, and the mechanism of suppression may well be translational.

E. Schierenberg (Max Planck Institute for Experimental Medicine, Göttingen)

reported progress in defining the complete embryonic cell lineage; this has now been described up to the 220 cell stage, and some lineages have been followed considerably further. The Göttingen group has also begun to characterise temperature sensitive lethal mutants that alter embryogenesis at the non-permissive temperature; some of these have abnormalities in the timing of cell divisions and in the movements of embryonic cells. The stages at which development is arrested have been described for these mutants, and also for a set of non-conditional lethal mutations isolated by P. Meneely (University of Minnesota, St Paul). Mutations altering post-embryonic cell lineages have been found: a number which affect vulval development were described by C. Ferguson (Massachusetts Institute of Technology). In two other mutants certain cells fail to undergo terminal differentiation and continue to divide in a stem cell mode instead (M. Chalfie, Cambridge, UK).

There is increasing interest in the experimental manipulation of development. Isolated blastomeres can now be cultured to the point where intestinal and muscle cells have differentiated (P. Bazzicalupo and J. Laufer, Boulder). The fate of the blastomeres is largely independent of the presence of their neighbours, and after blockade with cytochalasin and colchicine, characteristic intestinal granules develop only in those blastomeres that would normally give rise to the intestine. By both these criteria early development is mosaic. On the other hand, definite episodes of regulative development after hatching have been demonstrated by cell ablations with a laser microbeam (J. Kimble, Cambridge, UK) although here too development is predominantly mosaic. In further analysis of these events cell specific markers will be of great importance. Suitable probes are cloned cDNA preparations which can be used for *in situ* hybridisation to mRNA (K. Edwards, Boulder), antibodies to specific cell products (M. Klass, Boulder), monoclonal antibodies (Y. Argon, Carnegie Institution, Baltimore) and plant lectins (E. Hedgecock, Cambridge, UK).

Many other investigations were reported at the meeting but cannot be described here in detail, including work on ageing, cuticle structure, dauer larva formation, egg laying, fertilisation, hormones, intermediary metabolism, neuropharmacology, nutrition, sex determination, sperm motility, and tail formation. There were relatively few presentations on the neurobiology of *C. elegans*, although almost all of the wild type neuroanatomy has now been reconstructed. Work on the related but much larger nematode *Ascaris* was reported by J. Donmoyer and J. Walrond (University of Wisconsin, Madison); the organisation of the ventral

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*The Second International *Caenorhabditis elegans* Meeting, supported by the US National Institute of Aging and National Science Foundation was held on 10–13 May 1979.

cord neuroanatomy appears to be similar in the two species, and electrophysiological experiments (impossible in *C. elegans*) have led to the formulation of a model for bend formation in *Ascaris*. The same two classes of acetylcholinesterase are found in both nematodes, and both are widely distributed in *Ascaris* (C. Johnson, University of Wisconsin, Madison). The elimination by mutation of the two classes (but not either class alone) leads to defective movement in *C. elegans* (J. Culotti, Göttingen).

The participants seemed to be united in their enthusiasm for *C. elegans*, and their interest in all aspects of its biology. Specialisation has not led to fragmentation. □

Nonsolar planets and their detection

from David W. Hughes

I AM never sure which is the more surprising supposition — that our planetary system is of such extreme rarity that it might be unique in the Galaxy — or that planetary systems similar to ours exist in orbit around one in every four stars. There seems to be no inherent reason why Earth should not be the only home for living creatures, an assumption that obviously makes our search for extraterrestrial life futile. On the other hand planets may well be common. There seems to be no sharp distinction between binary and multiple star systems and stars with planetary companions. The masses of small stars in binary systems seemingly grade continuously down to the masses of planets. The obvious solution to this dilemma is 'look and see'. The discovery of nonsolar planets would provide a vital clue to the origin of the solar system, a subject which is bedevilled at the present by the fact that only one solar system can be studied in detail. Bracewell and MacPhie have reviewed the problems inherent in searching for nonsolar planets in a recent edition of *Icarus* (38, 136; 1979).

Astrometry is one of the existing techniques which, according to the authors, "with sufficient refinement may detect nonsolar planets". Plate scales of 15 arc sec per mm can be obtained with very long focus refracting telescopes and these enable very small angular displacements of stellar images to be detected. A year's observation gives a precision of 0.003 arc sec. The problem is exemplified by the fact that an observer of the Sun from a distance of 10 parsec would be looking for an epicycloid wiggle of amplitude about 0.0005 arc sec and period of about 12 yr just to detect Jupiter. Needless to say, many researchers regard the astrometric technique as already sufficiently refined to detect planets. Van de Kamp and

Lippincott of the Sproul Observatory have reported the discovery of invisible planets around Barnard's star, Epsilon Eridani and Ci 2354. Lalande 21 185 is also thought to have a planetary companion. It is interesting to note however that unlike the near circular orbits of our planets these objects seem to have high eccentricities, a factor they have in common with double stars.

Orbiting planets can also produce a sinusoidal variation in the stellar radial velocity. Observed in the ecliptic plane and from a distance, the amplitude of the variation for the Sun is 12 ms^{-1} , equivalent to a $3 \times 10^{-5} \text{ nm}$ shift in the 656.28 nm Fraunhofer line of the solar spectrum. Present practice achieves a precision of several hundred metres per second so this is a technique for the future.

When the combined light of Jupiter and the Sun is viewed from say 10 parsec only about one in 6×10^8 photons received actually comes from Jupiter. Also light from the Sun, diffracted by the edge of the telescope aperture obscures the planetary light. Diffraction may be overcome by shading the aperture, a process known as apodisation in which light transmission falls off gradually from the centre of the field towards the edge. Such a telescope, in orbit above the atmosphere can theoretically detect planets.

Infrared long baseline interferometry can be used. Planetary thermal infrared radiation might not be strong but at least it comes from near the peak of the spectral distribution. For stars, the infrared spectral region is well down from the peak of the spectrum. The ratio between stellar power and planetary power drops to around 5,000 at wavelengths longer than about $20 \mu\text{m}$. As the distance between the elements of an interferometer is increased, fewer and fewer objects can be detected. The ones that can are those with the smallest angular diameter and this of course favours planets. Bracewell and MacPhie consider possible infrared observations that can be obtained by future space probes. The first one entails having two orbiting infrared ($40 \mu\text{m}$ say) collectors separated by 44 km (that is 10^9 wavelengths). The angular diameter of the star is then about five times the interference fringe spacing so as the star changes its position relative to the fringe pattern the receiver power hardly varies. The planet however is about one tenth the stellar angular diameter and fringes sweeping over the planet produce a substantial modulation. Unfortunately the observing advantage gained is only about 20, which does little to reduce the previous factor of 5,000.

An improvement can be made by placing a minimum of the interference pattern on the star. The planet is then placed at the

adjacent maximum, this requiring, at $40 \mu\text{m}$, a base line of 7.7 m for an angular separation of 2.6×10^{-6} rad between star and planet. The star to planet power ratio drops to an amazing 1/80. This signal can be modulated, and thus more easily detected against the background, by spinning the interferometer about an axis through the star. Unfortunately the basic pointing accuracy required is 0.001 sec arc. For a detector of area 1 m^2 and a receiver of bandwidth $\Delta\lambda$ equal to 0.1λ a planet like Jupiter 10 parsec away would produce a flux of 2 photons per second. A reasonable signal to noise ratio is obtained with 10 h of observing time. Unfortunately again, the particles that scatter the zodiacal light produce a strong infrared background and the proposed detector would register 150 photon sec^{-1} from this source. To measure the planetary signal against this large background to an accuracy of a few per cent would take a month.

The device proposed by Bracewell and MacPhie has two off-axis paraboloids producing the interference null. Tracking control uses the interferometer optics but at visual wavelengths, the infrared and visual beams being separated by 45 degree plates. The critical components of the detector will be kept at liquid or superfluid helium temperatures, less critical parts having to make do with the 30 K temperature of the triple point of neon.

Obviously this instrument is utilising all the hoped-for technical improvements. It is also apparent that all our attempts to detect non-solar planetary systems rely so far on the observation of minor fluctuations in large quantities. We will have to wait a long time before we get a high definition view of another solar system, an agonising wait which does little to stifle our feeling of loneliness in the universe. □

Reconstructing the past

from Peter D. Moore

It is the task of the palaeoecologist to make mental reconstructions of past environments on the basis of such evidence as assemblages of fossils. First the assemblage must be interpreted in terms of contemporaneous communities of organisms; second, the environment is reconstructed from what is known of the ecology of the organisms involved. The Quaternary palaeoecologist would seem to have certain advantages at both these interpretive stages as most of the species with which he deals are still extant and can therefore be subjected to various types of community analysis and other types of ecological enquiry. Just how real these advantages are may be questioned, for

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many problems of interpretation remain at both levels of analysis.

These problems are well illustrated by the current concentration of attention on the closing phases of the last glaciation. Of particular interest is the climatic event which took place between about 11,000 and 10,000 yr ago, which involved the reversal of the trend towards increasing warmth and deglaciation, and resulted in the re-advance of glaciers in Scandinavia and Scotland and the reformation of local corrie glaciers in outlying mountainous districts. This event (often referred to as the 'Younger Dryas' after the discovery of remains of the plant species *Dryas octopetala* within sediments where the episode was first described), was very localised on a global scale.

Some views of the conditions prevailing during these times were expressed at a meeting of the Quaternary Research Association at University College, London in January. W. A. Watts (Trinity College, Dublin) showed that the biological changes induced by the event were pronounced and of both qualitative and quantitative character in the maritime fringes of North-West Europe, as in the western British Isles, but were weaker and became increasingly difficult to detect in fossil assemblages as one moved eastwards and southwards across Europe. In the area of the Alps there are only small, quantitative changes in flora during the Younger Dryas together with some sediment changes indicating increased erosion. South of the Alps, in the Po Valley, there are no sediment changes and the entire event is difficult to detect on the basis of the pollen record.

Such geographical variations in the fossil record of the time illustrate the local nature of some apparently profound climatic changes. In the case of the Younger Dryas event, it suggests that the climatic cause was associated with oceanic air or water masses, and Watts drew attention to the marine sediment evidence of a more southerly location of the Polar water mass front in Younger Dryas times.

Having established the geographical and temporal pattern of this climatic deterioration, one is then faced with the question of the precise sequence and nature of climatic conditions within its span.

Evidence from benthonic ostracods was presented by J.E. Robinson (University College, London) for the shelf waters of North-West Europe. Here the Younger Dryas event is marked by a decrease in species richness, which is now a characteristic of Arctic waters, and also by the occurrence further south of ostracod species now restricted to the Arctic Ocean, such as *Rabillimis mirabilis* and *Krithe glacialis*. This use of indicator species is an interpretive technique commonly used by palaeoecologists, which is based on the assumption that the factors which determine current distribution patterns of extant organisms are the same as those

which limited their distribution in the past. In this case temperature is thought to be the critical limiting factor. Although attractive as a rule of thumb, there are several problems associated with the use of this line of reasoning. Most physical environmental factors are complex in their diurnal and seasonal variations and it is only rarely that one can pinpoint the interaction between such a factor and the life history of an organism which results in the limitation of the organism's range. Also, such relationships are often made complex by the involvement of other species, such as prey, predators, parasites and competitors. Because such communal relationships can vary in time, the physical factors currently coinciding with an organism's range may not have so coincided in the past.

This is certainly not a problem confined to ostracods. Work on beetles presented by G.R. Coope and M. Joachim (University of Birmingham) has the same interpretive difficulties. They examined fossil assemblages of beetles from sediments at St Bees, Cumbria, which just predate the Younger Dryas. They showed a sequence of species moving from those with present distributions of a southern European type (occurring before 12,000 yr ago) to those with present ranges restricted to northern Scandinavia (occurring after 11,600 yr ago). They point out that, although this suggests increasing coldness of climate, two facts make precise climatic interpretations difficult. First, there are no modern equivalents of these beetle communities and, second, in times of rapid climatic fluctuation, as these undoubtedly were, stable communities in which competitive sorting had taken place were never established.

The plant fossil record is equally subject to these problems. J.B. Macpherson (Memorial University of Newfoundland) attempted an analysis of Younger Dryas climates on the basis of certain indicator pollen types. At a kettlehole site in the Spey Valley, Scotland, she found a pollen sequence in Younger Dryas sediments which led from an *Empetrum* maximum, through an *Artemisia* maximum, back to a further *Empetrum* peak. Together with evidence from sediment stratification, she interprets this as indicating a climatic sequence of oceanic, high snowfall, through continental, low precipitation, back to oceanic once again.

Empetrum was long ago used as an indicator of oceanicity by Jessen (*Proc. R. Ir. Acad. B* 52, 85; 1949) and its value in this respect has been discussed at length by Brown (*New Phytol.* 70, 841; 1971). The argument is based entirely on the present range of the genus and experimental data are lacking. Its modern distribution is complicated because it is found in continental areas (for example, Siberia), where it occurs mainly under coniferous forest canopies. It survives in sites of low winter temperature if there is adequate snow-lie. Many species of *Artemisia* do

have more continental distributions, but it is a large and ecologically varied genus and the assignment of a blanket 'continental' label is undoubtedly an oversimplification. It is not impossible that the balance between *Empetrum* heath and *Artemisia* herb communities could be determined by a factor such as wind speed and frequency, which would affect snow-lie.

W. Pennington (University of Leicester) reported the results of a quest for present-day equivalents of the lost plant assemblages of the Younger Dryas. European sites have proved of limited value because of the current high latitude extension of forest due to the oceanic influence of the Gulf Stream drift. Greenland, on the other hand, has very restricted forest and hence is a more likely site for the existence of plant communities resembling those of the late-glacial in their pollen rain spectra.

The coastal fringe of western and southern Greenland has a low rainfall (100-250 mm) and, growing on bare patches of soil, *Artemisia borealis* is locally frequent. The surface lake sediments from such areas have up to 4 or 5% *Artemisia* pollen, which approximately corresponds to the percentage cover of the plant in the catchment area. These values are similar to those found in Younger Dryas pollen assemblages in northern Scotland.

A recently published pollen diagram from Loch of Winless in Caithness, North-East Scotland by Peglar (*New Phytol.* 82, 245; 1979) has about 2-3% *Artemisia* during the presumed Younger Dryas, which falls to zero with the commencement of the Holocene (Flandrian). At the transition there is a peak of more than 5% *Empetrum*. If this diagram is compared with that most recently published from North Wales, Melynlyn in Snowdonia by Walker (*New Phytol.* 81, 791; 1978), a very similar sequence is found. *Artemisia* attains about 5% in the Younger Dryas and there is an expansion of Ericales (undifferentiated) at the opening of the Holocene. Evidently the changes of climate occurring over a short period at that time were more profound than those climatic differences between the two sites resulting from geographic, spatial factors at any given time.

The conclusion one must draw from these examples is that we are still far from being able to make precise statements concerning the climate of the Younger Dryas on the basis of biological evidence. The use of indicator species, even if adequate autecological information is available, can be misleading unless taken in the context of total assemblages. And the interpretation of whole assemblages is frustrated by the lack of precise present-day equivalents. □

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review article

Meteorology of the Indian summer monsoon

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The seasonally varying monsoon circulations associated with the annual heating and cooling of the Asian continent are the most important large-scale aspects of the general circulation of the atmosphere. The Indian summer monsoon affects the lives and the economies of many countries in Asia. The study of this phenomenon has been mainly limited to the Indian subcontinent whereas it takes place over the entire Indian Ocean. The lack of information over the marine region will be removed this year during the first Global Atmospheric Research Programme Global Experiment with the implementation of the Monsoon Experiment (MONEX) observational programme.

THE term monsoon has been used to describe the biannual complete reversal in the prevailing wind flow in the lower atmosphere over the Indian Ocean. The reversal of the air flow is mainly a consequence of the distribution of land and ocean characterised by a large continent in its northern part and a large ocean in its southern part (the topography is also important). The monsoon (south-west during the summer and north-east during the winter according to the direction of low-level flow) is primarily caused by differential heating between the continental areas and the oceans as a result of the zenithal march of the Sun. This was recognised in 1628 when Edmund Halley proposed that the monsoons of South Asia result from differential heating of land and sea surfaces. In 1921, Simpson¹ supported this hypothesis but also emphasised the role of the Himalayas.

The term monsoon does not, however, only apply to the wind regimes prevailing over the Indian Ocean, it also describes the phenomenon in the tropical and equatorial regions of Africa and South Asia. The Asiatic summer monsoon is the best known monsoon region, probably owing to the associated rainfall. Here the term monsoon refers to the rains which fall during the June–September period, although mariners usually associate the monsoon with winds.

The burst of the monsoon

As the Sun moves northwards bringing spring to the Northern Hemisphere, heat lows begin to form over the continental regions surrounding the Arabian Sea in May. A trough of low pressure forms and extends from Somalia northwards across Arabia and into the principal heat low located over Pakistan; this low-pressure belt extends northeastwards into central India. By the end of May, the heat lows have become well established and the south-west monsoon flow spreads northwards over the Arabian Sea, India and the Bay of Bengal. This change in the lower troposphere occurs quite abruptly and simultaneously over all the Indian Ocean: within a few days the intensity of trade winds of the Southern Hemisphere increases and the cross-equatorial flow becomes established². The rainfall is not synchronous with the dynamical changes. In a rainfall orientated

definition of the monsoon, the south-west monsoon sets in during late April and early May in Burma. The rainbelt then advances northwestwards over a broad latitudinal area reaching the Coromandel coast towards the end of May and the Thar Desert in early July (Fig. 1). This rain belt can be associated with the northwards motion of the ITCZ located over the head of the Bay of Bengal during the winter months. The advance is unsteady and is not evenly paced. At Delhi, for instance, the average date of monsoon arrival is 2 July, but between 1901 and 1950 the actual dates ranged from 17 June to 20 July, with a standard deviation of 7.8 days. The rainfall is so sudden and spectacular that it is known as the burst of the monsoon.

The upper air circulation also undergoes an abrupt change in the entire Northern Hemisphere. At the end of May, an elongated warm belt appears between the Arabian Desert and southwestern China along 28° N in the mid-troposphere. The westerly southern jet moves downstream into China and dissipates; it is replaced by an easterly jet. This jet appears first over the Bay of Bengal and expands eastwards to the Philippines and westwards to Africa. The mid-tropospheric anticyclone established during the pre-monsoon season disappears in June and is replaced at about 80° E by the upper trough moving from the Bay of Bengal. Over northern India and nearby, two anticyclones come into existence in June; one over the Thar Desert

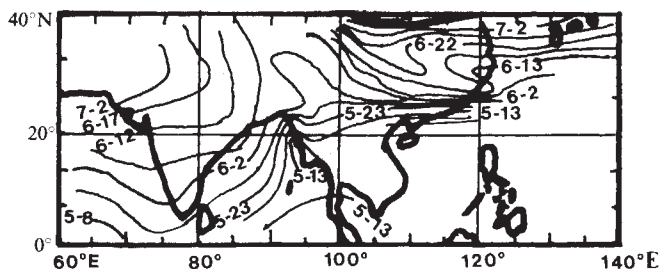


Fig. 1 Average onset dates of the rainy season (from ref. 57).

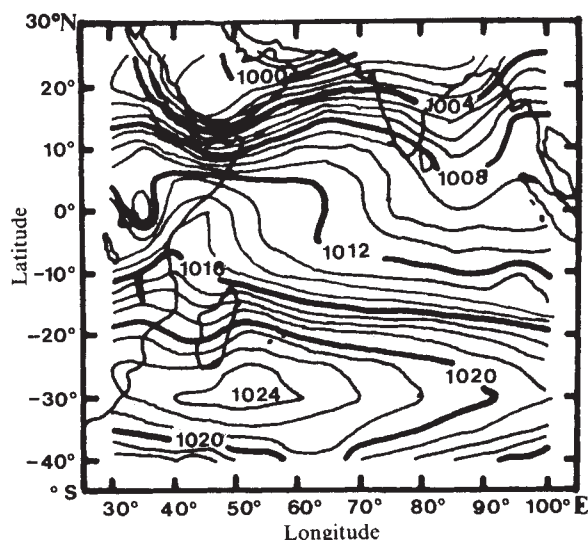


Fig. 2 July mean sea level isobars (mbar) (from ref. 58). The mascarene high pressure over southern Indian Ocean, the monsoon trough over India and the equatorial trough to the east of 70° E can be seen.

(above 700 mbar) and another over southern Tibet (above 500 mbar).

These changes in circulation patterns that take place in June are many and varied, and meteorologists have tried to relate the changes to the burst of the monsoon. Some have related the advance of the monsoon with the shift of the upper trough and the westerly jet and the development of the easterly jet³. Other meteorologists think the burst takes place when the air from the Southern Hemisphere goes into the circulation over the sub-continent⁴. In fact there seems to be no cause-effect relationship between the development of the circulation in the lower and upper levels, the insolation and the influence of topography being prominent in both cases. Another closely related question is what determines the year-to-year variations in the date of the onset of the monsoon.

Climatology of the Indian summer monsoon

As we have seen, certain elements are established over the Indian subcontinent during the pre-monsoon months. However, the Indian summer monsoon must be seen as a broad-scale system and it also includes components in the Southern Hemisphere. For a complete picture of the phenomenon all these elements must be described.

The monsoon trough over northern India is a very prominent feature of the synoptic weather charts during the summer season (Fig. 2). It is a part of the global equatorial trough of the northern summer season and indicates the separation between air of Northern and Southern Hemisphere origin⁵. It lies parallel to and about 450 km south-west of the Himalayas and is probably topographically anchored. The southwesterly to westerly air current from the Arabian Sea and the deflected air current from the Bay of Bengal meet along the Gangetic Valley giving rise to this trough which is connected to the Pakistan heat low. The topographical features of the subcontinent play an important combined dynamical and thermodynamical role in the location of the heat low and the development of the Gangetic Valley trough. The Himalayas force the monsoon current to ascend and thereby release latent heat. Thus, the mountains contribute significantly to making the monsoon circulation self-sustaining in the lower levels of the atmosphere and the Indian monsoon circulation different from monsoons in other parts of the world. The influence of topography on the streamflow also extends to higher levels. The heat low extends to above

850 mbar while the trough extends to about 500 mbar. Above 500 mbar the westerly and easterly flow is associated with the Tibetan anticyclone, to the north of 27° N, which has its maximum amplitude near 200 mbar.

Over the Arabian Sea the low-level flow from the Southern Hemisphere is the main characteristic of the monsoon. There has been controversy about the origin of the southwesterly winds⁶ although recent experiments have demonstrated that these winds effectively correspond to the south-east trades of the Southern Hemisphere deflected after crossing the Equator, by the Coriolis force and the Southern Hemisphere trough located around 80° E on the Equator. This was shown by the measurements of radioactivity over the Arabian Sea which allows us to separate land and marine air⁷ values. Another set of measurements was given by lagrangian trajectories of constant-level balloons flying in the boundary layer and launched from the Seychelle Islands (Fig. 3)⁸. The cross-equatorial flow is not uniform at all longitudes; it is weak in the eastern Indian Ocean where it enters the Bay of Bengal, and is strong along the East African coast (Fig. 4)⁹. Associated with the Southern Hemisphere trough on the Equator are weak westerlies found around 80° E and 5° S. Along the Somali coast, the air flow is characterised by one of the most intriguing phenomena of the monsoon: the low-level Somali jet which is in fact a major part of the southwesterly flow to the Indian continent (Fig. 5a and b)¹⁰. This low-level current, most pronounced at heights of 1–1.5 km, flows from the ocean near Mauritius over the northern tip of Madagascar to reach the Kenya coast at about 3° S. It then penetrates inland over the flat low-lying eastern areas of Kenya, Ethiopia and Somalia to reach the coast again near 9° N and then swings across the Arabian Sea to India. It splits into two parts over the sea, the more northern branch intersecting the west coast of India near 17° N while the southerly branch moves eastwards just south of India. This jet is occasionally reinforced or even temporarily replaced by a stream of air moving northwards up the Mozambique channel. The maintenance of the jet and its periodic fluctuations in strength are not well understood. A recent study using geostationary satellite images to determine the low-level air flow from cloud displacements¹¹, suggests that the variation in strength (occurring with a quasi-biweekly period) result through the influence of cold fronts at mid-latitudes in the Southern Hemisphere which induce surges in the south-east trades as suggested earlier¹². Its three-dimensional structure was studied in 1977 using an instrumented aircraft¹³. Jet-speed winds also occur over the central Arabian Sea and the Indian Peninsula in connection with the Somali jet¹⁴. The problem of the numerical simulation of this jet has been examined¹⁵. The β -effect, the orographic barriers over East Africa and Madagascar and a broad-scale monsoon forcing over India are some of the crucial elements for a proper simulation. Off the Somali coast, the atmospheric jet passes over the well known area of upwelling cold-coasted water which has also been numerically simulated^{16,17}.

The air mass over the Arabian Sea consists of an inversion layer which exists over the flat lowlands of eastern Africa and over most parts of the Arabian Sea, inhibiting the development of deep clouds¹⁸. The height of the base of the inversion layer, which probably separates the low-level deflected moist trades from drier continental air above, increases eastwards and becomes less marked. The inversion layer disappears east of about 65° E where low-level convergence and ascent in the troposphere favour the dominance of deep clouds and occasional development of monsoon disturbances. How the Western Ghats affect the destruction of the inversion is important¹⁹. This inversion layer is a control factor in the air-mass modification over the Arabian Sea and can affect the efficiency of monsoon heat engine.

Another important feature of the monsoon system is the upper tropospheric tropical easterly jet near 150 mbar^{20,21}. It has winds of roughly 80–100 knots and the strongest winds are found just to the west of the southern tip of India. Its intensity oscillates with a quasi-biweekly period (phase of generation and

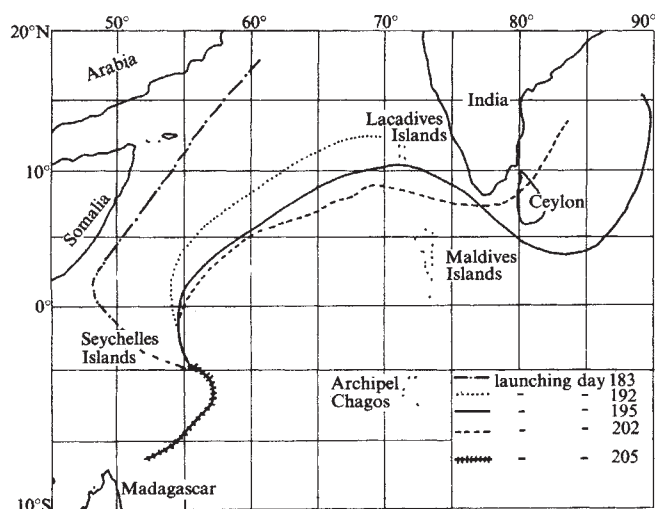


Fig. 3 Trajectories of constant-level balloons at 900 mbar, launched from the Seychelle Islands in 1975 (from ref. 8). Days are 1975 Julian days. The trajectory going towards Madagascar corresponds to a local reversal of the monsoonal flow due to the westward propagation of a tropical disturbance near 10° S (ref. 59).

degeneration early noticed by workers) and it must be seen as a part of the global upper air circulation in the tropics²². A numerical model of the broad-scale monsoon system with a few additions can simulate many of the well known broad features of the monsoon and particularly the easterly jet²³.

It seems difficult to define limits of the monsoon circulation. Reversal of flow in zonal and meridional direction from lower to upper tropospheric has led some authors to suggest a closed circulation. The inflow and outflow from the monsoon area are linked through the general circulation in other areas. With the advent of summer, an east-west Walker circulation with ascent over north-east India and descent over the Pacific and a north-south meridional circulation with ascent over north India and descent over equatorial regions are set up²⁴. The conditions in which the Walker circulation changes its intensity and location and the way in which it interacts with the monsoon circulation are largely unknown.

Monsoon depressions

Monsoon depressions are an important component of Indian weather and have been studied for several years. About 1–3 depressions form per month during the monsoon months, particularly in July and August. These depressions begin to be seen over the Bay of Bengal where satellite photographs show cloud clusters. They do not acquire hurricane intensity during the summer months, because they are not long over the oceanic areas before landfall and the large-scale vertical wind shear over the troposphere inhibits local accumulation of the latent heat release over the disturbance. Heavy rainfall associated with these depressions is the most outstanding associated phenomenon. Up to 300–400 mm of rain in 24 hours have been recorded. The structure of these perturbations has been studied from observations made once the depression has moved over central India where some upper air observations are available^{24,25}. The composite structure has also been studied²⁷.

The depression is an intense low-pressure system with a vigorous associated circulation; winds of up to about 50 knots have been noted (Fig. 6). Whereas the typical horizontal scale of the depression is known to be around 500 km in the formative stage, it is typically 1,500 km over land. The disturbance has a cold core with its vertical axis tilting eastwards with a slightly warm core above. The maximum rainfall rates occur to the west of the depression. The perturbation is confined to the lower troposphere from the surface up to 400 mbar. The strongest

activity is concentrated in a narrow vertical tube ahead of the depression, characterised by strong cyclonic activity, convergence and upward motion. Relatively weak activity is found behind the depression where anticyclonic vorticity, divergence and subsidence are noted. Case studies indicate that the disturbance is imbedded in a region where the conditions necessary for the combined barotropic–baroclinic instability to exist are satisfied. Monsoon depression is primarily maintained by cumulus convection. The cumulus convective heating generates eddy-available potential energy by releasing heat at appropriate levels above the cold core. Here the rising of relatively warm air contributes significantly to the generation of eddy kinetic energy of the depression.

Whereas early studies suggested *in situ* formation in the Bay of Bengal²⁸, recent analyses have led to a proposal of scale interactions of westward moving wavetrains. Such an interaction gives rise to a westward propagating downstream amplification²⁹.

Mid-tropospheric cyclones are also an important part of the south-west monsoon. They can be found in the northeastern part of the Arabian Sea and in northern Bay of Bengal. They are known either to move very slowly westwards or remain quasi-stationary for many days^{30,31}. The scale is of the order of about 3,000 km horizontally and 5 km vertically. Their largest amplitude is near the 600 mbar surface and they appear as a closed cyclonic circulation generally in the middle troposphere. They usually have a pronounced warm core above the middle level and a slightly cold core below that level. They are usually characterised by very intense convective and non-convective rainfall rates: total rainfall amounts of the order of 20 cm per 24 h can be noted. Flows near the surface and at the 200 mbar level do not show closed circulation. Despite case studies, detailed analysis of these depressions as well as numerical studies are necessary for a clear understanding of the development mechanism of the mid-tropospheric disturbances over the entire monsoon region.

The monsoon rains

A rapid build-up of atmospheric moisture over India augers the beginning of its monsoon season. Precipitable water vapour increases nearly twofold from March to June³² and this high water-vapour content of the monsoon air is largely responsible for the heavy rainfall in the summer. The summer rainfall is the main component of the annual rainfall over the subcontinent except in Kashmir and the extreme south of the Peninsula and the east coast area: nearly 80% of India's total annual rainfall falls during the months June to September³³. The orographic

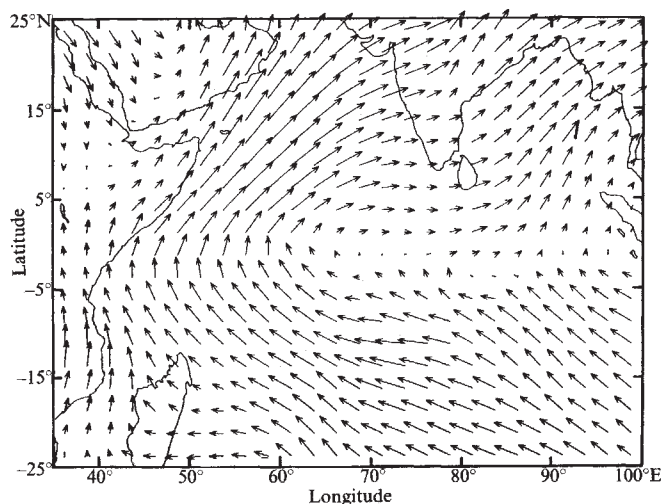


Fig. 4 Wind field over the Indian Ocean on 14 July 1975 as determined by an objective assimilation of ship reports (from Cadet and Reverdin, personal communication). The length of the arrow is proportional to wind intensity.

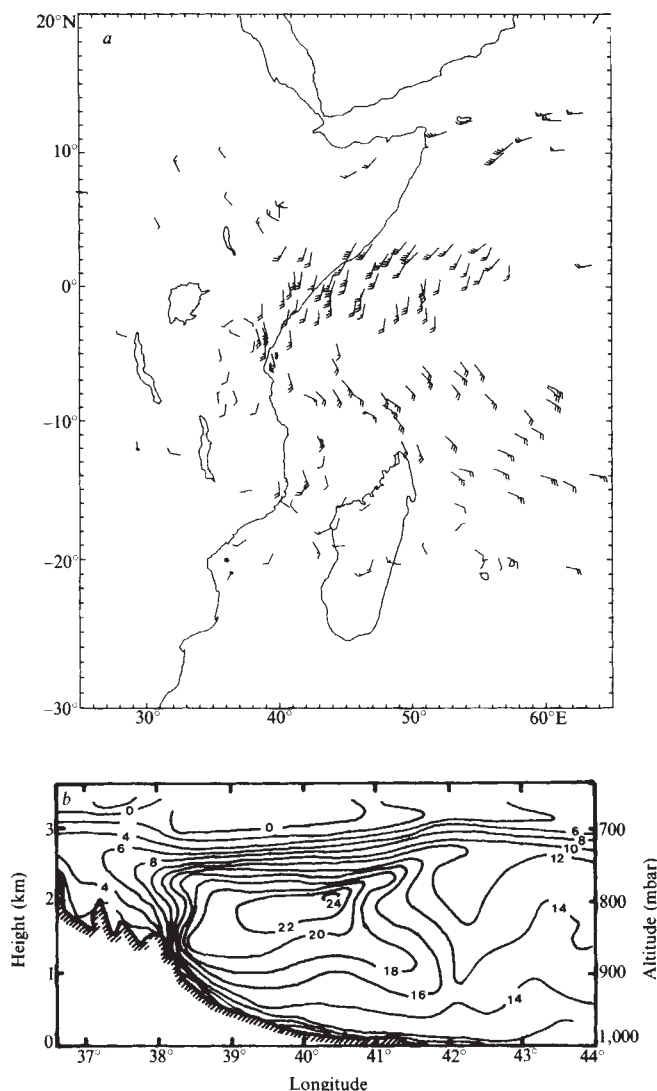


Fig. 5 *a*, Low-level wind vectors determined from cloud displacements on 23 July 1978 inferred by METEOSAT pictures (from ref. 11); *b*, vertical and longitudinal structure of the meridional (southerly) wind component along the Equator on early morning of 4 July 1975 (from ref. 13).

influence for producing strong convergence in the lower layer is important in the distribution of rainfall. The west coast of India receiving the southwesterly flow and the Burma coast affected by the Bay of Bengal branch of the monsoon receives considerably more rain than the lee-side of the Western Ghats and Burma Mountains. The important rainfall over central India is largely due to the influence of the depressions moving from the north Bay of Bengal and to some extent due to convergence at the trough and the southwards slope of the trough axis with height. The only region contrasting with the common view of heavy rains is the Thar Desert in the western half of northern Indo-Pakistan. The presence of the air mass inversion between the lower shallow moist current (about 800 mbar) and upper drier air mass³⁴ causes less rainfall in this area.

The low-level current over the Arabian Sea originates in the Southern Hemisphere. However, the origin of moisture contained in this southwesterly flow is still questionable. A large part of the moisture available over the Indian subcontinent is supplied by the Arabian Sea branch of the monsoon. Over India the major water-vapour influx takes place across the Trivandrum-Bombay section, and the layer from the surface up to 700 mbar contributes 86% (ref. 35). The onshore fluxes are well correlated with rainfall along the west coast although a small

fraction is converted into precipitation (13%)³⁶. While early estimations led to the conclusion that the contribution from cross-equatorial flow is less than that from the evaporation over the Arabian Sea, further computations have shown the existence of a certain water-vapour influx from the Southern Hemisphere³⁷. Some of the flux (30%) into the Indian subcontinent comes from the Southern Hemisphere. The maximum cross-equatorial moisture flux takes place along the East African coast and can be associated with the low-level Somali jet. Observations suggest that some relationship exists between rainfall in India and the Somali jet: increased cross-equatorial flow followed by increased rainfall over western India¹⁰. However, this question deserves careful consideration³⁸. Also, during a drought year the seasonal mean Somali jet is weaker than during a normal year²². An appreciable amount of moisture seemed to be picked up over the Arabian Sea. Thus, positive correlations have been found between sea-surface temperature over the Arabian Sea and monsoon rainfall over western and central India³⁹. Tests, made with a general circulation model, suggest that rains over India are sensitive to change in sea surface temperature over the Arabian Sea⁴⁰.

Monsoon rains exhibit considerable variations both from one year to the next and within one year (Fig. 7). Thus, the Indian summer monsoon has two basic synoptic patterns: the active and the break (or weak) monsoons. The succession of these patterns gives the well known pulsatory nature to the Indian monsoon which also manifests itself in the wind field. Thus, the monsoonal flow in the lower troposphere (below 500 mbar) undergoes rather drastic changes in association with rainfall variations: during active monsoon periods, a series of disturbances form and move west-northwestward across the Gangetic Plains and rainfall is widespread over the monsoon region. These interruptions, or breaks, last between 3 and 10 days (2–3 weeks in some years), and usually occur between the dissipation of one monsoon depression and the formation of another. During this phase of the monsoon, the monsoon trough shifts to the north from its normal position and heavy rainfall is concentrated along and near the foot of the Himalayas. Low-level westerlies shift north to the Gangetic Plains and in peninsular India they usually weaken and decrease in depth. The upper tropospheric easterlies spread to the north. During such epochs, when the monsoon trough is shifted to the foothills of the Himalayas, a secondary trough forms over the south Bay of Bengal and the south Arabian Sea near the Equator. Thus, there is a well marked negative correlation between the change in cloud density over central India and the equatorial Indian Ocean⁴¹. Feeble mid-tropospheric lows move from east to west in this trough. The monsoon can be revived under the influence of mid-tropospheric lows moving northwestwards along the west coast of India⁴² but there may be other ways of initiating this monsoon sequence^{43–46}.

Moisture distribution over the Arabian Sea is different during the two phases of the monsoon. During weak monsoon conditions, the moisture field is stratified whereas this stratification disappears during active monsoon and upwards transport of water vapour occurs. Evaporation seems to be larger during an active spell than during a weak one. During active monsoon, a part of the water vapour obtained by evaporation is transported towards the Indian subcontinent, the other being precipitated over the sea whereas during weak monsoon, the evaporation is used locally in the weather development⁴⁷.

Numerical simulations show that cold sea-surface temperature over the west Arabian Sea leads to a drastic reduction of monsoon rainfall over India because the cold sea decreases the evaporation rate and increases the surface pressure leading to a reduction in the cross-equatorial flow and moisture flux downstream⁴⁰. Thus analysis of a series of sea-surface temperatures over the Arabian Sea shows that during a weak monsoon there is a significant drop in sea-surface temperature over a large area compared with a period of strong monsoon⁴⁸. A numerical simulation experiment of the Somali jet shows that the cross-equatorial flow in the west Arabian Sea intensifies the jet as a

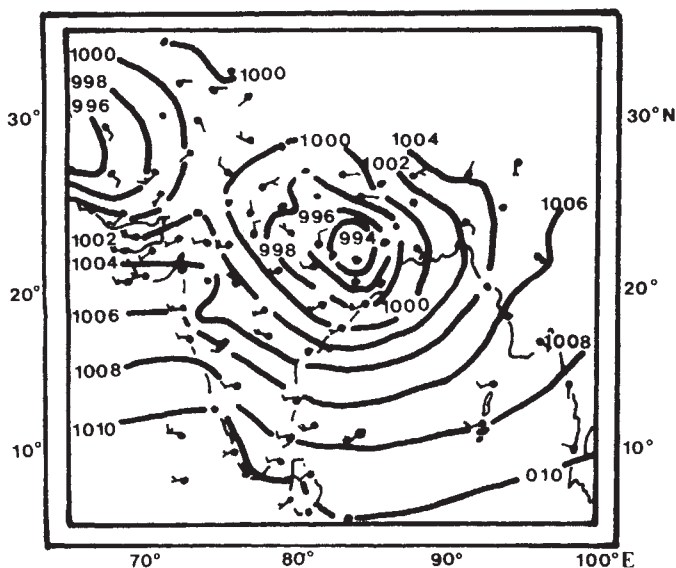


Fig. 6 Sea-level chart on 4 August, 1968 depicting a monsoon depression moving inland from the Bay of Bengal (from ref. 25).

result of the strengthening of the monsoon over India¹⁵. These and other experiments on the interaction between the Arabian Sea and the Indian monsoon suggest that the strength of the Indian monsoon does not depend on the strength of the cross-equatorial flow; this contrasts with other results that demonstrate a controlling influence of the Somali jet on the monsoon.

Active and break monsoons should be viewed as part of the fluctuations occurring on a very large scale and the changes in circulation of the Indian subcontinent might result from distant effects. Synoptic patterns have to be found which could characterise a break and an active monsoon⁴⁹. The partition of the monsoon into two phases may be compared with the quasi-biweekly oscillation of the broad-scale monsoon elements. The two-week period may be divided into an active and a less active one and our study suggests an interesting ordering of the phase of the various elements of the monsoon system⁵². In fact, no unique or detailed description exists for active and break monsoon and numerical simulation may be useful for understanding the phenomenon and for separating the effects of the different synoptic components.

Long-term variability of monsoon rainfall has been studied in some detail by Indian workers. Quasi-biennial oscillation (QBO) in rainfall has been found at several stations in India⁵⁰ as well as in the annual rainfall in some meteorological subdivisions of the country and the monsoon rainfall as a whole⁵¹. Recent investigations using data of ISMEX-73 indicate that certain interactions exist between QBO and sea-surface temperature and monsoon currents in the equatorial area of the Indian

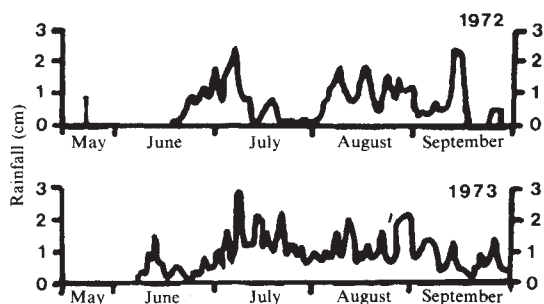


Fig. 7 Daily rainfall over central India (75°–85° E to 15°–25° N during a drought year (1972) and a near-normal rainfall year (1973) (from ref. 22).

Ocean⁵². This result suggests that there is some interaction between the circulation in the tropical stratosphere which exhibits the well known QBO for zonal winds. There does not seem to be a significant relationship between Indian monsoon rain and solar activity⁵¹.

The retreat of the monsoon

The monsoon in the Indian subcontinent begins to withdraw in mid-September when the circulation pattern in the Northern Hemisphere changes from a summer to a winter regime. The weakening of the easterlies, the southward shift of the monsoon trough and the appearance of the westerly jet stream in northern India are all reversals of the events that occur during the burst of the monsoon. In a year when a break takes place in September, the monsoon may retreat prematurely. The equatorward-motion of the ITCZ (inter-tropical convergence zonal) brings abundant rainfall during the retreat of the monsoon⁵³.

Numerical simulation

The main objective of monsoon studies is to improve the ability to predict the atmospheric motion and associated rainfall over Asia. Empirical attempts have been made to predict, for example, the onset of the monsoon from wind activity over the Arabian Sea⁵⁴ or rainfall of western India from surges and lulls in the cross-equatorial flow⁵⁵. Studies made to determine dynamical and thermodynamical structure of the atmosphere during break and active monsoon have been tentatively used to predict the occurrence of these different phases of the monsoon.

The development of numerical simulation is at an early stage but will be activated after the results of the Monsoon Experiment in 1979. Different types of numerical models can be developed. Specific regional fine-mesh models can be used to study certain components of the monsoon such as the Somali low-level jet, Bay of Bengal depression and mid-tropospheric disturbances. Global models with nested fine-grid system must be used to determine the conditions under which monsoon circulation interacts with the global circulation. Predictability experiments are urgently needed to investigate the feasibility of numerical weather prediction over the monsoon region for developing numerical prediction models.

The experimental programme to study the Indian summer monsoon

For a long time, observations have been confined to the Indian Ocean although their deficiencies were recognised. The Indian Ocean is so large that international cooperation is necessary to make marine observations. An international experiment, the International Indian Ocean Expedition (IIOE), was conducted in 1963–64 and produced a large amount of information about the development of the monsoon over the Indian Ocean⁵⁶.

The idea of a special monsoon experiment to be conducted during the First GARP Global Experiment (FGGE) was proposed in 1971. This idea was developed through two experiments: ISMEX-73 and MONEX-77. ISMEX-73, conducted by USSR and India, took place between 15 May and 10 July 1973. MONEX-77 took place during the summer of 1977 for the study of the Indian summer monsoon, and the winter of 1977 for the study of the winter monsoon over the Malaysia-Indonesia region. These two limited experiments considerably increased our knowledge of the development of the monsoon over the Indian Ocean and the structure of some monsoon elements (monsoon depressions and Somali low-level jet). They prepared the way for the main experiment to be conducted in 1979.

The main experiment, MONEX-79, has two components: the winter monsoon and the summer monsoon. The winter monsoon was conducted during the First Special Observing Period (SOP I) of FGGE (15 January–15 March). The summer monsoon will take place from May to September with an intensive period during SOP II of FGGE (May–June)⁵⁷. The

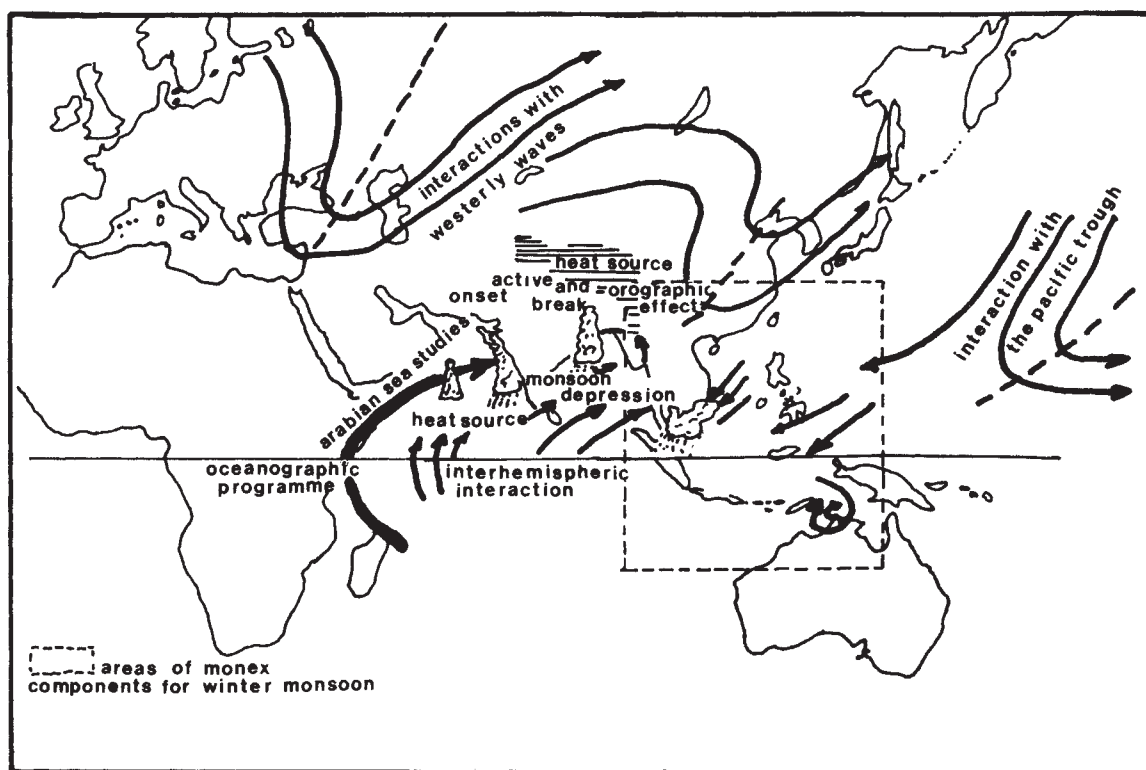


Fig. 8 MONEX-schematic illustration of major component programmes (from ref. 57).

summer MONEX can be divided into two phases. The first coincides with the SOP II and, taking advantage of the ship network over this area, will study the development of the monsoon over the Arabian Sea (Fig. 8). Instrumented US aircraft will study the structure of the atmosphere. About 90 superpressure constant-level balloons will be launched by a French group from the Seychelle Islands and Diego Suarez (Madagascar) to fly in the tropical boundary layer. The balloons tracked by the TIROS-N satellite will provide, along with their positions, recordings of pressure, temperature and moisture at flight level. This experiment will complement the boundary layer data by giving lagrangian trajectories of the mixed layer over the ocean. At the beginning of July the ships will move to the Bay of Bengal for the second period to study monsoon disturbances generated over this region. For the entire period (May to September), the surface synoptic network has been strengthened. Commercial aircraft and ships will enhance the network (especially the aircraft which will provide data on upper tropospheric winds over the ocean). The space-based part of the global observing system will be very important, especially the geostationary satellite over the Indian Ocean (GOES-1) from which winds can be determined by cloud tracers. Thus, with the high resolution geostationary satellite picture adequate low-level winds over the Indian Ocean can be deduced and a daily description of the circulation obtained. An important oceanographic programme will also be carried out during MONEX.

Outlook

From the MONEX and FGGE data sets obtained in 1979, it will be possible to study the Indian summer monsoon on both a regional and a global scale. Undoubtedly the knowledge of the phenomenon will be improved within a few years. All this information is necessary for the development of reliable numerical models for weather forecasting. Such numerical forecasting of the vagaries of the Indian summer monsoon would benefit the populations of southern Asia. However,

forecasting needs continuous information about the present state of the atmosphere. The large marine extension of the phenomenon means this can only be made on an operational basis by space observatories; hence polar orbiting and geostationary satellites are needed.

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articles

How tidal heating in Io drives the galilean orbital resonance locks

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Tidal heating in Io is the most likely energy source of its volcanic activity. Tidal dissipation in Io and Jupiter also controls the resonance configuration among the three inner satellites. The formation of the several resonance locks is described in detail in this article. This model sets limits on the Q values of both Io and Jupiter.

THE Voyager 1 spacecraft fly-by of Jupiter provided startling images of the several active volcanoes on Io. Tidal heating in Io has been identified as a potentially important energy source¹ and it has been suggested that this same mechanism also controls the evolution of several orbital resonance locks among the three inner satellites. This article discusses in more detail how these resonance locks are maintained and describes the effects of dissipative tides in both Jupiter and Io on their establishment and evolution. The most significant results of this study are that the three-body resonance may have formed $<5 \times 10^8$ yr ago and that a measurement of the rate of tidal heating in Io not only determines, say, the Q value of Io, but also Q_J of Jupiter. Arguments are also given which limit Q_J : $2 \times 10^5 < Q_J < 2 \times 10^6$. The factor $Q/2\pi$ is equal to the ratio of the peak strain energy in each tidal flexing cycle to the energy dissipated per cycle⁶.

The most well known resonance involves the three inner galilean satellites, Io (1), Europa (2), and Ganymede (3). The mean longitudes λ of these three satellites when combined in the following combination: $\lambda_1 - 3\lambda_2 + 2\lambda_3$ happens to equal an odd multiple of 180° . Lieske² found that the libration amplitude of this resonance variable is $0.066^\circ \pm 0.013^\circ$ and that the period is 2,074 d. The other set of resonance variables involve the near commensurability of the mean motions of the inner pair and the forced precession of their pericentres, $\tilde{\omega}_1$ and $\tilde{\omega}_2$. The mean daily motion of $\tilde{\omega}_1$ and $\tilde{\omega}_2$ is $-0.7395^\circ \text{d}^{-1}$. Sinclair³ and Chao⁴ noted that the arguments $\lambda_1 - 2\lambda_2 + \tilde{\omega}_1$, $\lambda_1 - 2\lambda_2 + \tilde{\omega}_2$ and $\lambda_2 - 2\lambda_3 + \tilde{\omega}_2$ executed small amplitude librations about either 0 or 180° . In fact, it is just the interaction of the last pair of variables which predominately controls the three body couple.

Table 1 contains some of the pertinent physical data^{2,10}: mass M , radius R , semi-major axis a , mean daily motion n and both free forced eccentricities. The free or proper eccentricity

represents that eccentricity which would remain if we could slowly remove all perturbing forces. The libration amplitude of the 2:1 resonance is equal to the ratio of the free to the forced eccentricity.

Tidal interaction on Io

It is a common misconception that the principal tide on a synchronously rotating satellite moving in an eccentric orbit is radial. This idea can be traced to MacDonald's⁵ purely phenomenological model which tried to incorporate the restriction that tidal dissipation in a synchronously locked satellite cannot transfer angular momentum from its spin to its orbit. In reality, the dominant tide moves back and forth across the mean sub-planet point as the satellite moves in its orbit (see Fig. 1). At pericentre, where dissipation and the resultant torque are greatest, the orbital angular motion is $n(1+2e)$. As seen from the planet, the satellite seems to rotate retrograde with angular velocity $\sim -2en$. Dissipation in the satellite causes a time delay ($\approx 1/Qn$) of high tide leading to a displaced tidal bulge which leads the instantaneous sub-planet point. The torque by the planet acts to spin up the satellite but is prevented from doing so because of the existence of a permanent bulge. The effect of dissipation is to displace the rigid bulge until the resulting torque by the planet exactly cancels the average tidal torque.

The equations describing the evolution of the satellite orbit due to the inelastic planetary and satellite tides are⁶:

$$\frac{1}{n} \frac{dn}{dt} = -c(1 - (7D - 12.75)e^2) \quad (1)$$

Table 1 Physical data of the three inner galilean satellites

	Io	Europa	Ganymede
$M/M_J \times 10^5$	4.684 ± 0.022	2.523 ± 0.025	7.803 ± 0.030
n (deg per day)	203.4890	101.1747	50.3176
a (km)	422,000	671,400	1,071,000
$e_{\text{forced}} (2:1)$	0.0041	0.0101	0.0006
e_{free}	$1 \pm 2 \times 10^{-5}$	9×10^{-4}	0.0015
R (km)	$1,818 \pm 3$	$1,533 \pm 27$	$2,608 \pm 32$

$$\frac{de^2}{dt} = -\frac{2}{3}c(7D-4.75)e^2 \quad (2)$$

$$D = 2k\left(\frac{R}{R_J}\right)^5 \left(\frac{M_J}{M}\right)^2 \frac{Q_J}{Q}; c_{10} = 6.1 \times 10^{-13} Q_J^{-1} \text{ s}^{-1} \quad (3)$$

For small homogeneous satellites the Love number $k \approx 3/19 \rho g R \mu^{-1}$ where ρ is the density, g the surface gravity and μ the rigidity. If Io were lunar-like then $\mu \approx 6.5 \times 10^{11}$ and $k = 0.027$. Incidentally, Jupiter's fluid Love number equals 0.50. The parameter D is the ratio of the tidal scale factor of Io to that of Jupiter. For Io we find that $D \approx 1,300$ if we adopt 'plausible' values for $Q_J = 5 \times 10^5$ and $Q_{10} = 100$. We shall argue later that $D \approx 4,300$.

Suppose that Io were formed well inside the orbit of Europa about 4.6×10^9 yr ago. Any initially free eccentricity in Io's orbit would be quickly damped by Jupiter, and Io's orbit would thereafter expand, driven by the dissipative tide it raises on Jupiter. Europa's orbit would also expand, but because of Io's greater mass and smaller semi-major axis, Io would spiral out faster. Io would eventually approach the 2:1 commensurability with Europa. This near resonant interaction is adequately described by the following set of differential equations^{4,7}.

$$\frac{dn_1}{dt} = 3 \frac{M_2}{M_J} n_2^2 \left(\frac{a_2}{a_1}\right)^2 [e_1 C_{11} \sin(V_1 + \tilde{\omega}_1) + e_s C_{21} \sin(V_1 + \tilde{\omega}_2)] + \frac{dn_1}{dt_T} \quad (4)$$

$$\frac{dn_2}{dt} = -6 \frac{M_1}{M_J} n_2^2 [e_1 C_{12} \sin(V_1 + \tilde{\omega}_1) + e_2 C_{22} \sin(V_1 + \tilde{\omega}_2)] \quad (5)$$

$$\frac{dp_1}{dt} = -in_2 \left(\frac{a_2}{a_1}\right)^{1/2} \frac{M_2}{M_J} C_{11} \exp(iV_1) - \left(\frac{7}{3} Dc + i\dot{\omega}_{s1}\right) p_1 \quad (6)$$

$$\frac{dp_2}{dt} = -in_2 \frac{M_1}{M_J} C_{22} \exp(iV_1) - i\dot{\omega}_{s2} p_2 \quad (7)$$

where $V_1 = \lambda_1 - 2\lambda_2$. The coefficients $C_{11} \approx C_{12} = C_1 = -1.18$ and $C_{21} \approx C_{22} = C_2 = +0.42$ are polynomials in a_1/a_2 . The variations of the pericentre $\tilde{\omega}$ and eccentricity are most easily described by the variables $p = e \exp(-i\tilde{\omega})$ and $q = p^*$. The parameter $\dot{\omega}_s$ is the mean precession rate of the orbital pericentre due to the jovian oblateness, satellite interactions, and so on. For Io, $\dot{\omega}_s \approx +0.16^\circ \text{ d}^{-1}$. This term and the tidal acceleration of Europa shall be ignored in the following discussion—Europa's acceleration is unimportant because the coefficient c_2 is small ($\approx 0.021c_1$) while the ratio $D_2 \approx 1,000$.

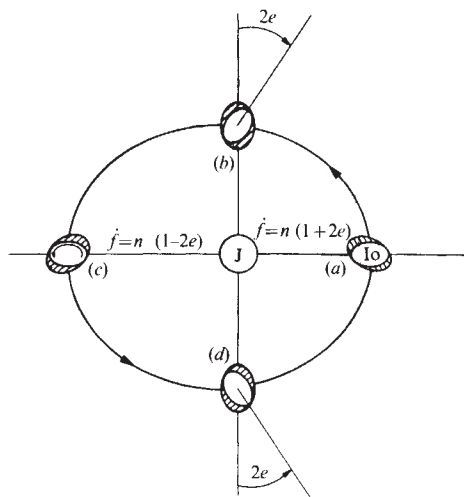


Fig. 1 Location of tidal bulge of a synchronously rotating satellite at different points in its orbit. The tide lags behind at pericentre (a) where the orbital angular velocity $\dot{\phi}$ is greatest. The optical libration of the permanent bulge has the opposite phase and is $2e$ radians behind at point (b) and ahead at point (c).

A solution for the forced motion of p can be obtained by assuming that $V_1 = \nu_1$ is constant. This approximation is valid as long as either ν/n is large compared to $(eM_1/M_J)^{1/2}$ or the free eccentricity is small compared to the forced eccentricity, both of which happen to hold in this case. We find

$$p_1 = -\frac{M_2}{M_J} \frac{n_2}{\nu_1} \left(\frac{a_2}{a_1}\right)^{1/2} C_1 \exp(iV_1 + \delta) \quad (8)$$

$$p_2 = -\frac{M_1}{M_J} \frac{n_2}{\nu_1} C_2 \exp(iV_1) \quad (9)$$

The mutual perturbation leads to a forced retrograde motion of the pericentres of both orbits. Dissipation in Io induces a phase lag $\delta = \frac{7}{3} Dc/\nu_1$ in Io's pericentre. This phase lag means that Io is slightly closer to Europa E just after than just before conjunction (see Fig. 2). Thus the torque, when averaged over the conjunction phase, tends to accelerate Europa in its orbit and decelerate Io.

Substituting the above solutions for p_1 and p_2 into the equations (4) and (5) for $\dot{n}_1$ and $\dot{n}_2$, we obtain

$$\frac{dn_1}{dt} = 7cn_1 D e_1^2 + \frac{dn_1}{dt_T} = -cn_1(1 - 14De_1^2) \quad (10)$$

$$\frac{dn_2}{dt} = 14cn_1 D \left(\frac{a_1}{a_2}\right)^2 \frac{M_1}{M_2} e_1^2 \approx -10.3cn_1 D e_1^2 \quad (11)$$

As Io tidally evolves outwards, Io's forced eccentricity increases until it reaches the critical value $\approx 1/(35D)^{1/2} \approx 0.0026$. Europa's limiting eccentricity is ~ 0.0014 . Thereafter, the relative outward acceleration of Europa causes its mean motion to be exactly half that of Io. This stable state is maintained until Europa encounters the 2:1 commensurability with Ganymede. The 4:1 commensurability of Io with Ganymede is third order in the eccentricities and represents a considerably weaker interaction. The inelastic tide raised on Europa will repel Ganymede. Still, the acceleration of Europa by Io is so great that Europa's forced eccentricity would have to be at least three times larger than its present value (0.0101) for this mechanism to push Ganymede out so that $\dot{n}_2 = 2\dot{n}_3$. Before Europa's eccentricity can be pumped up to this value the 2:1 frequency $\nu_2 = n_2 - 2n_3$ of the outer pair approaches that of the inner pair, $\nu_1 = n_1 - 2n_2$. The vanishing of the difference frequency describes the presently observed three-body resonance.

Coupling between Europa and Ganymede

The near resonant gravitational couple between Europa and Ganymede can be obtained by indexing the subscripts of (4–7) by one. The resulting distortion in the shape of Europa's orbit by both Io and Ganymede is

$$p_2 = -\frac{M_1}{M_J} \frac{n_2}{\nu_1} C_1 \exp(iV_1) - \frac{M_3}{M_J} \frac{n_2}{\nu_2} \frac{a_2}{a_3} C_2 \exp(iV_2) \quad (12)$$

When this solution is substituted back into the differential equation for n_1 , n_2 and n_3 , we obtain the interaction involving the resonance argument $\phi = V_1 - V_2$.

$$\frac{d^2\phi}{dt^2} + An_2^2 \sin\phi + cn_1(1 - 45.0De_1^2) = 0 \quad (13)$$

$$\frac{d\nu_1}{dt} + 0.68An_2^2 \sin\phi + cn_1(1 - 34.6De_1^2) = 0 \quad (14)$$

The coefficient $An_2^2 \approx -0.03858 (1 + 0.19 \cos\phi)^\circ \text{ d}^{-1}^2$ if we include contributions from higher order effects (J. Lieske, personal communication).

The secular acceleration of ϕ and V_1 vanish when Io's forced eccentricity $\approx 1/(13D)^{1/2}$. As this eccentricity is 1.5 times larger than that necessary to maintain the 2:1 Io–Europa lock, this implies that the system passes through the capture phase (that is, when ϕ vanishes and reverses sign) with the smaller eccentricity. After the three body lock is established, Io's eccentricity is pumped up until the quasi-stationary state is achieved.

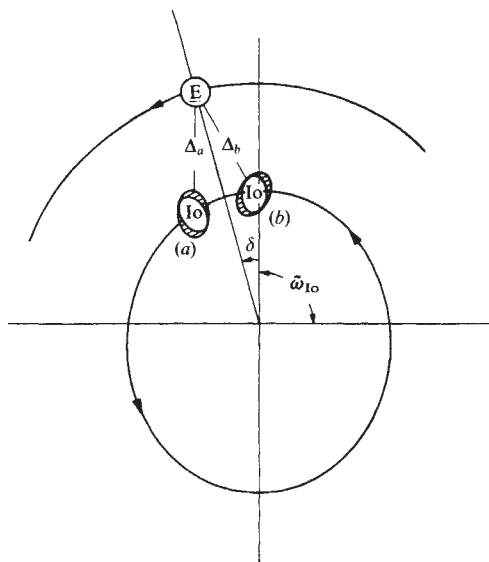


Fig. 2 Relative location of Io–Europa at equal time intervals before (b) and after (a) conjunction. Because of the tidal offset δ in Io's pericentre, $\Delta_b > \Delta_a$ and the average tangential torque repels Io from Europa.

The torque acting to repel Ganymede results from an offset of the resonant argument ϕ from 180° . Actually, the transfer of momentum from Europa to Ganymede involves the same kind of mechanism outlined for the 2:1 case, except that in this case the offset in the forced motion of Europa's pericentre by Ganymede is coupled directly to the offset driving the Io–Europa pair. The next problem to resolve is the capture mechanism.

One mechanism for changing the energy and the libration amplitude of a pendulum-like equation is through the addition of a $\dot{\phi}$ term. The dominant $\dot{\phi}$ term in this case comes from the resonance-induced variation of Io's forced eccentricity which is inversely proportional to $\nu_1 = n_1 - 2n_2$. From analysis of the equations of motion (4–7, 13, 14) we find that near the three-body resonance the variations are $\delta n_1 \approx 0.125\dot{\phi}$, $\delta n_2 = -0.276\dot{\phi}$ and $\delta n_3 = 0.023\dot{\phi}$. The additional term to be added to the left hand side of equation (13) is therefore

$$+60.8cDe_1^2n_1\nu_1^{-1}\dot{\phi} \quad (15)$$

The coefficient of $\dot{\phi}$ is positive, implying that the librational energy is reduced through this term. The resulting differential equation describing the motion and tidal evolution of the three-body resonance happens to be nearly identical to that of spin-orbit resonance^{8,9}. An estimate of the probability that capture into resonance occurs can be obtained from comparing the change in the kinetic energy during the cycle that $\dot{\phi}$ reverses sign to the maximum change per cycle. The resulting capture probability P_c is given by

$$P_c = \frac{2}{1 + \pi/4[1 - 45De_1^2]/60.8De_1^2\nu_1^{-1}A^{1/2}n_2} \quad (16)$$

Evaluating this expression with the appropriate values of e_1 , ν_1 and A at capture we find $P_c \approx 0.90$. Capture into the three-body resonance is almost certain.

The damping of the libration amplitude ϕ_m is approximately described by⁹

$$2 \sin \frac{1}{2}\phi_m \approx \left(\frac{16}{\pi}\right)^{1/2} \left(\frac{A(0)}{A(t)}\right)^{1/4} \exp\left(-\frac{1}{2} \int \sigma(t) dt\right) \quad (17)$$

where $\sigma = 60.8cDe_1^2n_1\nu_1^{-1}$. Because forced e increases from $1/(35D)^{1/2}$ to $1/(13D)^{1/2}$ the coefficient σ varies from $\sim 250c$ up to $1,100c$. The secular decrease of ν_1 can be obtained from averaging equations (13) and (14). The result is

$$\frac{d\nu_1}{dt} = -0.32cn_1(1 - 12.5De_1^2) \quad (18)$$

Given that $\phi_m = 1.1 \times 10^{-3}$ (0.066°), we find that the time since capture $\Delta t \approx 0.024c^{-1} = 1,200 Q_J$ yr. Furthermore, the relative change in Io's semi-major axis is only 0.7%. On the other hand, the present forced eccentricity $\approx 1/(13.8D)^{1/2}$ is very near the limiting value of $1/(12.5D)^{1/2}$. From this we can deduce that $D \approx 4,300$. Limits on Q_J can be obtained from a comparison of the rate of tidal heating to that expected from radiogenic sources and an estimate of the total heat input during the lifetime of the three-body resonance.

From equating the rate of change in Io's orbital energy by the satellite tide to the tidal heating rate dE_1/dt , we obtain

$$\frac{dE_1}{dt} = \frac{1}{3} M_1 n_1 a_1^2 \frac{dn_1}{dt} \left(\text{Io} \right) = \frac{7}{3} M_1 n_1^2 a_1^2 c D e_1^2 \quad (19)$$

The resulting estimate for the rate of heating is $3.0 \times 10^{25} Q_J^{-1} \text{ erg s}^{-1}$. This is to be compared with the radiogenic value of $5 \times 10^{18} \text{ erg s}^{-1}$ for a body of Earth-like composition. The apparent fact that Io is much more active than the Earth suggests that $Q_J \ll 2 \times 10^6$, if we assume that for a given heat input that surface activity scales with radius.

The total heat deposited in Io per gramme of material during the three-body resonance lifetime is only $0.9 \times 10^{10} \text{ erg g}^{-1}$ or 260 cal g^{-1} . If no heat is lost, the mean temperature rise is $\sim 900 \text{ K}$, which is enough to partially melt Io.

A lower bound of Q_J of $\sim 2 \times 10^5$ and an upper bound of the heat input in Io of $\sim 10^4 \text{ cal g}^{-1}$ can be obtained from the condition that Io has significantly tidally evolved and that the 2:1 resonance lifetime is the age of the Solar System. The tidal heating of Europa is at most $\sim 100 \text{ cal g}^{-1}$ because of its very small eccentricity (≈ 0.0014) during the 2:1 resonance lifetime.

Voyager 1 images of Europa (fly-by distance $\sim 750,000 \text{ km}$) revealed long linear features that may be due to tidally induced fracturing. The relative age of these features may be deduced from the much more detailed Voyager 2 images of Europa (fly-by distance $\sim 190,000 \text{ km}$; resolution $\sim 5 \text{ km}$). If indeed these features are of tidal origin, then they should have been produced in the past $10^3 Q_J$ yr during which Europa's forced e has increased from 0.0014 to 0.0101.

It should be emphasised that all of the above conclusions are predicated on Lieske's solution for the libration amplitude and, of course, the story changes if this measurement happens to be spurious. The major change is that the three-body resonance may be very old. The lower bound on Q_J is then reduced to $\sim 8 \times 10^4$ while the heat deposited in Europa could be as high as 10^4 cal g^{-1} because of its much larger forced e once the three-body resonance is established.

In the expansion of the Sun–satellite gravitational interaction, a forcing term exists with argument $2\lambda_1 - \Omega_3 - \psi$ and a period of 2,076 d which is very near the libration period of $2,074 \pm 10 \text{ d}$ (J. Lieske, personal communication). Ω_3 refers to the node of Ganymede's orbit and ψ to the node of Jupiter's equator to the jovian orbit. The amplitude of the forcing term is very sensitive to the difference in these periods. The determination of whether this observed libration amplitude is in reality due to this forced term rests primarily in improving the masses of the galilean satellites.

On the other hand, a more sophisticated tidal analysis which includes the third harmonic tides on Jupiter, european tides and higher order contributions to the coefficient of the $\dot{\phi}$ term are not expected to change the primary conclusion that the three body was established recently nor alter the above numerical estimates by more than $\sim 20\%$.

There are other observables which can be used to estimate the rate of dissipation in Io. Since Io is about the same distance from Jupiter as the Moon from the Earth, the maximum recession of Io should be no more than that found for the Moon ($\sim 3 \text{ cm yr}^{-1}$) and is completely negligible. On the other hand, the variation in Io's longitude times its semi-major axis is quadratic in time. For a rate of 1.5 cm yr^{-1} , the magnitude is 150 km (Δt^2 (in cent.) and might be detectable using $\sim 100 \text{ yr}$ old eclipse data.

Other possibilities are polar wander (thought to be driven by mantle convection on Earth) and a slightly faster than synchronous rotation. This latter possibility occurs if the non-hydrostatic component of the moment of inertia difference $(B - A)/C$ is small compared with the average, periodic tidal component. The minimum time scale for either effect is set by the relaxation time $\sim \eta h / \rho g R^2$ of the zero frequency tidal bulge. If the viscosity $\eta \approx 10^{22}$ St (like the Earth's asthenosphere) and the crustal thickness $h \sim 100$ km, this time scale is ~ 100 yr. Thus these effects are unlikely to be observable from image analysis of the Voyager 1 and 2 fly-bys which are separated by only 154 d.

Other implications

Some variants of this sequence of events can be dismissed because of their low probability. It is unlikely that Io first encounters the three-body resonance well before the 2:1 near commensurabilities are achieved, because the capture probability, which scales like $\nu^{-7/2}$, would be small. The possibility that Europa might have tidally evolved into a quasi-stationary 2:1 commensurability with Ganymede first limits the heat input in Io to ~ 200 cal g^{-1} . This amount of heat is probably insufficient to drive the observed surface activity.

Another variation is that Io's forced eccentricity, when Io encountered either the 2:1 or three-body resonance, may have been pumped up significantly larger than its present value. This would be expected if Io were initially more rigid than at present because of the absence of a large fluid core. The nearly elastic periodic tides would rapidly build up until the elastic limit is reached after which massive satellite-wide fracturing occurs. The catastrophic increase in tidal dissipation and perhaps tide height could provide the trigger for core formation.

Among the other satellite resonance locks, only the saturnian pair Enceladus-Dione has an e -type lock where the free eccentricity of one member is exceptionally small. The forced eccentricity of Enceladus is 0.0044 while the free eccentricity is $\sim 1 \times 10^{-4}$. Enceladus is apparently a small icy satellite ($M = 1.5 \times 10^{-7} M_s$, $R \approx 270$ km and $\rho \sim 1.0$ g cm^{-3}). The parameter $D \sim 60$ if we assume $\mu \approx \mu_{ice} \sim 9 \times 10^{10}$ and $Q_s/Q_{En} = 10^3$. Thus it is unlikely that this pair has reached a steady state configuration. It is possible that originally e_{En} was pumped up to some critical eccentricity which caused catastrophic fracturing. The effective rigidity may have been sufficiently reduced, such that this pair too has achieved a quasi-stationary orbital configuration. The Voyager 2 fly-by of Enceladus may reveal another curious satellite whose past and present state is controlled by tidal friction.

Conclusions

We conclude that tidal dissipation in Io is a plausible energy source for the volcanic activity and is the controlling mechanism which leads to the establishment and the present state of the several resonance locks. The most plausible sequence of events is in four stages. (1) All three satellites are in orbits far from either the 2:1 commensurabilities or the three body lock. The tide raised on Io quickly damps down the free eccentricity on a time scale $\leq 10^5 Q_1$ yr. Only modest tidal heating in Io occurs. (2) The dissipative tide raised on Jupiter by Io is dominant and causes Io's orbit to spiral outwards. No significant tidal heating occurs. Io approaches the 2:1 commensurability with Europa in stage (3). Io's forced eccentricity rapidly increases to the critical value $\sim 1/(35D)^{1/2} \sim 0.0026$. Thereafter the resonant interaction causes Europa's orbit to expand at exactly half that of Io's orbit. Tidal heating probably leads to the formation of a fluid core. Finally (4) Europa approaches the 2:1 commensurability with Ganymede but instead of dissipation in Europa repelling Ganymede we find that Io must work even harder using the three-body resonance to transfer angular momentum from Europa to Ganymede's orbit. We again rapidly reach a steady state as e_{Io} approaches $1/(13D)^{1/2}$.

Based on the observed three-body resonance amplitude of 0.066° , we have concluded that the three-body resonance was established 1,200 Q_1 yr ago. About 220 cal g^{-1} could have been deposited by tidal heating during this interval and is just sufficient to produce a large fluid core in Io. The 2:1 near commensurability of Io may be considerably older than the three body lock. Upper and lower limits of Q_1 are 2×10^6 and 2×10^5 . Analysis of Voyager data should provide an estimate of the energy necessary to drive Io's volcanic activity and provide a firm estimate of the jovian Q_1 .

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A twin-jet model for radio trails

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Radio trails are interpreted in terms of the twin-jet theory of double radio sources. A simple dynamical model of a narrow jet bending under the ram pressure of the intracluster medium is calculated and applied to some observations of the source associated with NGC1265. Observed radio emission must come from the interior of the jet, which is surprisingly stable to disruptive instabilities.

RADIO TRAILS (head-tail sources, twin-tail sources) are a distinctive morphological class of weak extragalactic radio sources^{1,2}. The most natural interpretation of their radio shape is that the associated active elliptical galaxy, which would otherwise have produced a normal double source, belongs to a cluster and is moving at $\geq 1,000$ km s^{-1} through an intracluster gas¹. The radio-emitting regions are then swept backwards by ram pressure. The wide-angle trails (bent doubles²) such as 3C465^{3,4} are an intermediate category where the motion of the galaxy

causes distortion to a lesser degree. The two best studied radio trails are 3C83.1B⁵⁻⁸, associated with the galaxy NGC1265 in the Perseus cluster, and 3C129^{5,6}.

In many weak radio sources, there is now good morphological evidence^{2,9,10} for continuous energy supply, via collimated jets or beams emanating from the nucleus of the associated galaxy or quasar^{11–13}. Recently, Owen *et al.*⁸ have produced a high resolution map of NGC1265 in which narrow, quasi-continuous streams can be seen emerging from the nucleus of a galaxy associated with a radio trail. These streams have a transverse angular scale $\leq 2''$ and appear to be bent back into a curved arc until, at a distance ~ 30 kpc from the nucleus (assuming a distance to the cluster of 100 Mpc), the jets disappear in high surface-brightness features and presumably merge into the trail. The radio emission from the beam at ≤ 5 GHz is $\sim 10^{40}$ erg s⁻¹, only a few per cent of the radio luminosity of the tail.

As argued by Owen *et al.*⁸ these observations pose difficulties for the ‘discrete blob’ model of radio sources^{14–16} in which independent gas clouds plough through previously undisturbed intergalactic medium. The distinctive feature of a jet interpretation is that each element of gas moves along a pre-established track (whose curvature is determined by the mean momentum discharge averaged over the recent history of the source) rather than on a trajectory depending on its individual cross-section and inertia. In NGC1265, the fact that there is a bright unresolved nuclear source indicates continuing activity and outflow from the galactic centre.

Here we give a simple discussion of the dynamics and equilibrium of a curved jet, assuming that the jet carries a steady flux of energy and momentum, and ignoring all complexities stemming from instabilities, entrainment, and so on. Then we make some quantitative estimates for NGC1265, and discuss the relationship between the curved jet and the extended tail. Further implications and various difficulties are also discussed.

Dynamics of a curved jet

Consider a narrow, collimated, supersonic jet emerging from the central region. It must come into dynamical equilibrium with the pressure of the cluster gas sweeping past the jet. The flow pattern that we envisage is shown in Fig. 1. The intracluster medium (ICM) of electron density n_e , temperature T_e , flows towards the jet with the galaxy's velocity with respect to the centre of the cluster, $\mathbf{V}_g$. We ignore gas motion in the ICM and assume that there is no significant mixing between the jet and the ICM, justifying this assumption *a posteriori*. If the galaxy moves supersonically, there will be a cylindrical stand-off bow shock; the stagnation pressure at the origin, where the jet is perpendicular to $\mathbf{V}_g$, will exceed the ambient ICM pressure by $p_0 = 2.9M_g^2 n_e k T_g$ where M_g is the Mach number associated with the galaxy's motion through the ICM¹⁷. (This formula is also approximately valid for subsonic motion). The jet will be accelerated transversely within this transition region and the density profile will adjust to establish a pressure gradient across the jet, and to come into pressure equilibrium with the ICM either by expanding through a series of rarefaction waves or through being heated by a combination of weak shocks and compression waves. Downstream from the jet, the flow will come into rough pressure equilibrium with the ambient ICM and so the pressure difference across the jet will be roughly the same as p_0 .

The rate of bending of the jet is governed by the jet density profile. In order to calculate a simple model, we treat the jet velocity V_j as constant, and the jet fluid as having a temperature T_j which depends on distance along the jet. We approximate the complex gas flow by relating the pressure at the jet surface to the stagnation pressure by the factor $\cos^2 \phi$, where ϕ is the angle between the surface normal and the surface normal at the stagnation point¹⁸ (see Fig. 1). This relates the pressure to the momentum flux incident on the surface in the shocked ICM. In cross-section, the jet profile then has the form $y = 2h \cos^{-1} [\exp(-(x/2h))]$ where the scale height is $h = 2 k T_i / m_{\text{pg}}$.

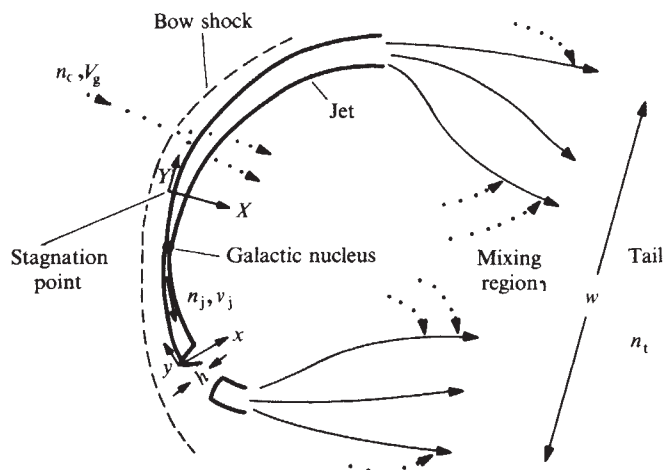


Fig. 1 Schematic diagram of the bending of a jet by a transverse supersonic flow. Axes X, Y describe the equilibrium shape of the jet in a frame moving with the galaxy. Axes x, y describe the profile of the jet in cross-section. The pressure difference across the jet is comparable with the ram pressure of the intracluster medium. In the jet, this is opposed by the centrifugal force associated with the curved trajectory. After bending through a large angle, the jets disrupt and share their mass, momentum and energy fluxes with the shocked intracluster medium. Dissipation of relative kinetic energy creates a mixing region of transverse size $\sim w$. The mixture of thermal gas, relativistic particles and magnetic field flows downstream away from the galaxy to form the l

where g is the effective acceleration felt by the material in the jet as it follows its curved path.

We make a similar assumption about the variation of the pressure p along the jet axis. In a frame such that X and Y are the coordinates of the jet relative to the point where the velocity is perpendicular to the galaxy's velocity, the jet curvature is $Y''(1 + Y'^2)^{-3/2}$. For a given effective specific heat ratio γ in the jet we can calculate the trajectory from

$$g = V_j^2 Y'' (1 + Y'^2)^{-3/2} = \frac{p_0}{n_{i0} m_n h_0} (1 + Y'^2)^{(1/2)\gamma-1} \quad (1)$$

The stagnation pressure, jet electron density and scale height are p_0 , n_{j0} and h_0 respectively. The scale height varies along the jet as $h = (1 + Y'^2)^{1/2} \gamma h_0$. From equation (1) we see that the jet Mach number M_j is related to the radius of curvature R_0 at the stagnation point by¹⁹

$$M_i = (R_0/\gamma h_0)^{1/2} \quad (2)$$

Note that if n_c and V_g are known, and h can be estimated from the radio contours, then the momentum flowing along the jet, $\pi h_0 R_0 \rho_0$, can be inferred from the observations (apart from projection effects). The energy flowing along the jet is larger by the unknown factor $V_i/2$.

Application to NGC1265 (3C83.1B)

The galaxy NGC1265 has a radial velocity $\sim 2,300 \text{ km s}^{-1}$ relative to the mean velocity of the Perseus cluster. Then

$$\begin{aligned} V_g &\sim 2,300 \text{ sec } i \text{ km s}^{-1}; \\ M_g &\sim 2.1(T/5 \times 10^7 \text{ K})^{1/2} \text{ sec } i \end{aligned} \quad (3)$$

where i is the angle between the galaxy's velocity (relative to the cluster centre) and our line of sight and T is the temperature of the ICM. If NGC1265 is bound to the Perseus cluster, then $\cos i \geq 0.6$. n_e is probably $\sim 3 \times 10^{-4} \text{ cm}^{-3}$; the X-ray data suggest $T_c \sim 5 \times 10^7 \text{ K}$, implying that M_g lies between 2 and 4, and vindicating our assumption of supersonic flow.

The internal pressure within the beam at the stagnation point is

$$p_0 \approx 3 \times 10^{-11} (n_c / 3 \times 10^{-4} \text{ cm}^{-3}) \text{ sec}^2 i \text{ dyn cm}^{-2} \quad (4)$$

An important check of the self-consistency of this model for NGC1265 is then to ask whether this value of p_0 permits a high enough volume emissivity to account for the observed radio emission. This emission is non-uniform along the jet, the mean value being $\sim 5 \times 10^{-12}$ dyn cm $^{-2}$. This value is calculated on the assumption that the spectrum extends, with spectral index 0.8, up to 10 GHz (see ref. 8), but making no allowance for relativistic protons. In the individual 'hot spots' the pressure is higher; the precise value depends on the detailed geometry, but an estimate would be $\geq 4 \times 10^{-11}$ dyn cm $^{-2}$. The corresponding equipartition field is in the range $(1-4) \times 10^{-5}$ G. Comparing these two estimates of the pressure we conclude that the magnetic and relativistic electron components of the jet fluid must be in approximate equipartition, and the thermal particles (though they may greatly outnumber the relativistic particles) cannot dominate the pressure. Furthermore, the radio emission cannot come from a region (for example, a thin surface layer or ribbon) involving some small fraction of the cross-section assumed on the basis of cylindrical symmetry.

The synchrotron lifetime for an electron emitting at 5 GHz in the equipartition field is $\sim 5 \times 10^6$ yr which is of the order of 10 times the time for the ICM medium to flow past the jet and probably shorter than the flow time along the jet. This argues in favour of emission from the jet itself rather than from a sheath of shocked ICM and indicates the need to reaccelerate relativistic electrons *in situ*. The most surprising implication of this model, however, is the small fraction of the jet kinetic energy that is dissipated along its length. The precise relationship between the radio emissivity and the jet geometry obviously depends on very specific details of the model. In almost every hypothesis, however, the transverse extent of the radio contours would correspond at least to a scale h . The narrowness of the observed radio jet then implies that $(h/R) \leq 0.05$, so that $\leq M_j^{-2} = 0.05$ of its kinetic energy can have gone into internal energy. Unless, for example the jet is a powerful millimetre source, it is impossible that a large fraction of the jet kinetic energy be radiated away, because the total observed jet luminosity is ≤ 0.02 of the radio power of the tail. These considerations in turn imply that the fraction of the ICM material impacting on the beam which can be entrained must be $\leq M_j^{-2} (V_g/V_j)$.

There is therefore observational support for the simple dynamical model of the jet outlines above. We have integrated equation (1) for a specific heat ratio of 5/3 (using $\gamma = 4/3$ makes little difference). The resulting flow must be projected onto the sky. We have used $\cos i = 0.8$ and varied the angle j between the jet axis and the line of sight to obtain a rough fit to the shape of the observed radio contours. This is shown in Fig. 2. We find that $j \sim 60^\circ$. If the jet angular width near the galaxy is δ , then $M_0 \sim 6(\delta/l'')^{-1/2}$, and the existence of unresolved features $\leq 0.5''$ in size suggests that $M_0 \geq 8$. However, if we take the width of a smooth trajectory enveloping the faintest contour, then we obtain $M_0 \sim 4$. The angle between the jet axis and the galaxy's velocity is $\sim 80^\circ$, and it is the closeness of this to 90° that is perhaps responsible for the unusually symmetric appearance of NGC1265.

Polarisation observations can in principle provide information on the density and hence V_j . The observations of Owen *et al.*⁸ do not provide polarisation data but if the reported 30% polarisation⁷ is due to the narrow jet, it implies highly ordered internal field structure, probably aligned along the jet by shearing motions. If the thermal plasma is uniformly distributed throughout the jet and not contained in dense clouds, then lack of depolarisation implies that $n_i \leq 0.06$. This is not a very significant limit. However, if the same level of polarisation in the narrow jet persisted down to lower frequencies it would set a more stringent limit on n_i , and thereby set a lower limit on V_j . If, for instance, the jet remained highly polarised down to 400 MHz, n_i would have to be so low that the required momentum flux could be supplied only if V_j exceeded $\sim 10^4$ km s $^{-1}$ (a value that seems likely on other grounds).

The total luminosity of the tail is $\sim 6 \times 10^{41}$ erg s $^{-1}$. There is unlikely to be sufficient kinetic energy flux associated with the



Fig. 2 VLA map of 3C83.1B at 4,886 MHz reproduced from Owen *et al.*⁸; only contours in the vicinity of the jet have been drawn. The shaded ellipse represents the half power contour of the clean beam, corresponding to a resolution of $1.5'' \times 1.0''$. Superimposed on this map is a trajectory obtained from integrating equation (1).

ICM to supply this. If this energy were all supplied by the jet then even assuming 100% efficiency we would infer, from our estimate of the momentum flux, a lower bound on V_j of 4,000 $(\delta/l'')^{-1} (n_e/3 \times 10^{-4} \text{ cm}^{-3})^{-1/2}$ km s $^{-1}$. The jets appear (at least on one side) to break up after bending through $\sim 75^\circ$. The jet material may then share its momentum with the shocked ICM downstream (M.C. Begelman, in preparation), the energy thereby dissipated going into the production of a sheared magnetic field and into particle acceleration. The magnetic field strength within the tail region (assuming equipartition between the thermal gas, relativistic electron and magnetic energy, and pressure balance with the ambient ICM pressure) is $\sim 10^{-5} (n_e/3 \times 10^{-4} \text{ cm}^{-3})^{1/2} (T_e/5 \times 10^7 \text{ K})^{1/2}$ G: lower by a factor $\sim M_g$ than the field strength in the jet and slightly greater than the equipartition value inferred from observations. The tail has a width $W \sim 40$ kpc and is highly polarised with a rotation measure ~ 30 rad m $^{-2}$. If the magnetic field is aligned parallel to the tail, as is expected if it is amplified by shear, then we can estimate the tail electron density to be $n_t \sim 10^{-4} \text{ cm}^{-3}$. For comparison, the mean electron density in the jet plasma is only $\sim 10^{-6} (\delta/l'')^{-1} (V_j/10,000 \text{ km s}^{-1})^{-1} \text{ cm}^{-3}$ and so there must be some mixing with the ICM. In any case, n_t is smaller than n_e ; hence this model supports the idea that buoyancy is important in shaping the outermost parts of the tail^{16,20}.

General implications

Our basic starting point has been to assume that the tail of NGC1265 is energised by a 'twin-jet' emerging from the nucleus. This assumption is supported by the smooth arc-shaped curvature manifested in the high-resolution radio maps, the existence of an active radio nucleus in NGC1265, and by analogies with normal double sources. This interpretation requires that the jets be surprisingly stable and resistant to Kelvin-Helmholtz instabilities: the intergalactic wind flows across the beam with very little mixing or entrainment. The jets are able to maintain a Mach number ≥ 4 whilst being curved through an angle $\sim 70^\circ$. We would conjecture that the real flow pattern has no sharp velocity discontinuities: small-scale instabilities would certainly be inhibited if the shear layer actually had a thickness comparable with h .

We note also that the density profile across the jet is unlikely to follow the simple exponential law that we have assumed in our explicit model. Moreover, the flow pattern of the intergalactic medium through the galaxy is presumably much more complex than the uniform wind we have considered; even if there is not a large 'dead zone' of gas trapped in the galaxy's potential well, the flow pattern is likely to be modified by the entrainment of gas

resulting from stellar mass-loss in the galaxy. Our estimates of the energy flux and momentum flux along the jets are, fortunately, insensitive to these details. From the power requirements in the tail, we can guess that $V_j \approx 10^4 \text{ km s}^{-1}$. This corresponds to a flow time along the jet of $2 \times 10^6 \text{ yr}$, a power $\sim 2 \times 10^{42} (n_c/3 \times 10^{-4} \text{ cm}^{-3})(\delta/1'')(V_j/10^4 \text{ km s}^{-1}) \text{ erg s}^{-1}$ and a mass discharge $\sim 0.05(n_c/3 \times 10^{-4} \text{ cm}^{-3})(\delta/1'')(V_j/10^4 \text{ km s}^{-1})^{-1} M_\odot \text{ yr}^{-1}$. The energy and mass fluxes required of the nucleus are very small compared with those of the most powerful doubles.

The irregularities in the radio contours delineating the jet path imply either that its properties are unsteady or that there are instabilities along its path. The latter would not be unexpected—what seems astonishing is that the jet is stable enough to persist at all when exposed to a strong transverse force. Variations in the central energy supply—involving changes in V_j or in the internal properties of the ejecta—will also lead to nonuniformities along the track of the jet. If the jet were ever to switch off, the channel which it had made would persist for a time $\sim 3h/V_j \sim 10^6 \text{ yr}$. Variations on timescales less than this would lead merely to inhomogeneity but if the nuclear activity revived after a dormant phase longer than this, the jet would need to be re-excavated; a bow shock would advance out at a speed $\sim M_j V_g$ as in the standard model for a double radio source component.

We have shown how the high resolution observations of NGC1265 may be interpreted in the context of a jet model and have constructed a simple equilibrium gas flow to describe the overall morphology. It remains to be shown that this flow is not subject to serious disruptive instabilities until the jet has bent through an angle $\geq 70^\circ$. The best hope for investigating

empirically the importance of these instabilities is perhaps with laboratory wind-tunnel experiments.

Finally, we note that, although other radio trails may not show such symmetric arc-shaped central structure when studied with $\sim 1''$ resolution,²¹ the same qualitative picture can apply. NGC1265 must be unusual in that not only do its velocity and beam axis both have substantial components transverse to the line of sight, but, furthermore, the beams happen to be directed almost perpendicular to the galaxy's motion through the IGM.

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Sequence, structure and activity of phosphoglycerate kinase: a possible hinge-bending enzyme

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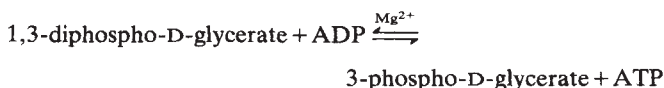
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The fitting of sequenced peptides to a high-resolution X-ray map of phosphoglycerate kinase has yielded the complete sequence and structure of the horse muscle enzyme. Metal ADP and ATP substrates are bound to one of the two widely separated domains in an environment that seems unsuitable for phosphoglycerate binding. The most plausible binding site for the phosphoglycerate substrate is on the other domain about 10 Å from the ATP, which implies the possibility of a large scale hinge-bending of the domains to bring the two substrates together in a water-free environment for catalysis.

PHOSPHOGLYCERATE KINASE (EC 2.7.2.3; PGK), catalyses the high-energy phosphoryl transfer reaction:



The enzyme is required for ATP generation in the glycolytic

pathways of aerobes and anaerobes, and for carbon fixation in plants. PGKs isolated from a wide variety of sources are monomeric with molecular weights around 45,000, with comparable amino acid compositions and similar catalytic properties. This has led to the proposal¹ that the enzyme has a highly conserved molecular and active site structure.

Preliminary X-ray studies of the horse muscle^{2,3} and yeast enzymes^{4,5} have shown that they are structurally homologous. The most remarkable feature of the PGK molecule is that its single polypeptide chain is organised into two widely separated domains of almost equal size. The results we report here suggest that this feature of the tertiary structure is probably associated with a large-scale 'hinge-bending' conformational change in the enzyme that is broadly similar to that previously reported for hexokinase²².

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Experimental methods

Initially, a three-dimensional electron density map of horse PGK was obtained by conventional isomorphous replacement, using two derivatives, ethylmercuric phosphate and ethylmercuric chloride, each of which react with up to six of the enzyme's seven sulphhydryl groups. The X-ray intensities of the native enzyme and the two derivatives were collected on a five-counter, five-circle diffractometer⁷ to 2.5 Å resolution. The data were corrected for Lorenz polarisation and radiation damage, and for absorption using the Huber-Kopfmann technique⁸. The phases of the 13,000 structure factors were estimated using the isomorphous and anomalous contributions of the heavy atoms. In parallel with the incorporation of the side-chain information from the sequenced peptides (see below), the protein structure was subjected to crystallographic refinement using the Hendrickson-Konnert constrained least squares program⁹, and the resultant calculated phases combined with the isomorphous phases using Hendrickson's method¹⁰ to produce improved electron density maps. This procedure was carried out in three stages using: (1) main-chain atoms only (including β -carbons); (2) main chain + 200 available side chains; (3) main chain + 400 available side chains. As shown in Table 1 at each stage the conventional *R* factor for the refinement decreased, the figure-of-merit of the combined phases increased, and the new electron density maps improved markedly in interpretability.

The sequence analysis was carried out using both automated and manual Edman degradation of cyanogen bromide peptides and fragments derived from them by digestion with proteolytic enzymes. Treatment of horse muscle PGK with cyanogen bromide produced the 14 peptides consistent with the presence of 13 methionine residues in the molecule. The cyanogen bromide peptides were separated by gel filtration, ion-exchange chromatography, and where necessary, paper electrophoresis. The sequences of the individual peptides were obtained by a combination of automated Edman degradation using a Beckman 890C Sequenator, and the dansyl-Edman technique¹¹. As with the enzymes from yeast, rabbit muscle¹² and human erythrocytes¹³, horse muscle PGK was found to have a blocked N-terminus. Thermolytic digestion of the N-terminal cyanogen bromide peptide revealed an *N*-substituted serine derivative, which was shown to be *N*-acetylserine by mass spectrometry.

Alignment of the sequenced cyanogen bromide fragments was carried out on the X-ray map. The six peptides containing more than 30 residues were fitted first. Their possible location on the molecule was determined using the following criteria: (1) each peptide began and ended at plausible methionine residues in the map; (2) any cysteine residues present in the sequence were located near one of the six known sites of mercurial binding; (3) Phe, Tyr and Trp residues in the sequence coincided with large side-chain densities; and (4) each of the 12 internal β -strands in the map corresponded to consecutive sequences of

four to six hydrophobic residues. The remaining eight peptides, which had 20 or fewer residues, often did not fulfil a sufficient number of criteria for an independent fit to be made with much confidence. Instead, when the sequence or lengths of all of them were available, they were fitted into the gaps between the longer peptides. Each fit was considered confirmed only when, in addition to all other criteria, it was found that internally positioned side-chains corresponded almost without exception to hydrophobic residues, and there was a good match of size and shape of the sequenced residues to their electron densities.

The 14 peptides were accommodated on the X-ray map in an end-to-end fashion, covering the complete polypeptide chain visible in the map. This fit is shown in Fig. 1, with the methionine residues marked to indicate the linkage of the sequenced peptides. The total of 416 residues in the polypeptide chain of horse muscle PGK corresponds to a molecular weight of 44,519, in good agreement with independent determinations¹; and the total numbers of each type of amino acid residue are consistent with the overall amino acid composition¹⁴. Preliminary comparison of this sequence with peptide sequences from yeast PGK suggests a considerable degree of homology between the enzymes (L. A. Fothergill, personal communication).

Molecular structure

Figure 2 shows a drawing in the conventional arrow and cylinder form of the tertiary structure of phosphoglycerate kinase. The division of the molecule into two discrete, separated globular domains can be seen clearly. It is now evident that the domains correspond to the N-terminal and C-terminal halves of the polypeptide chain, with the sole exception that the final 12 residues of the chain, forming helix 15, are packed in the N-terminal domain. There are therefore two links between the domains; the major one represented by residues 186–189 that form the junction between β -strand F and helix 7; and the minor one around residues 404–406 that connects helices 14 and 15. It is interesting to note that no element of regular secondary structure crosses the domain interface: strand F is clearly part of the N-domain, whereas helix 7 is associated with the C-domain; helices 15 and 14, respectively, are similarly disposed.

The N- and C-domains are almost precisely the same size and each is composed of a central β -sheet of six parallel strands surrounded by helices. In topological terms the order of the β -strands in the C-domain (CBADEF) is the same as that in the NAD-binding domain of the dehydrogenases¹⁵; whereas the order in the N-domain (CDBAEF) differs only in the location of strand D. There are, however, no significant similarities in the sequences of the two domains and their detailed structures are different. As in other glycolytic enzymes⁶ there is a marked tendency for the secondary structural elements to be organised as repeating (β - α) units¹⁶: in PGK the (β - α) motif is repeated 12 times.

In total, the PGK molecule has 15 helices, 12 internal β -strands, and at least five surface β -strands. On the basis of the number and extent of the secondary structural elements shown in Figs 1 and 2, the PGK molecule has a minimum of 104 residues located in β -structures, and 174 in helices: these numbers represent 25% and 42% respectively of the residues in the molecule.

Substrate binding

Horse PGK crystals grown in ammonium sulphate and equilibrated in potassium tartrate bind AMP and the complexes of ADP and ATP with Mg^{2+} or Mn^{2+} , at a single site on the C-terminal domain. The difference maps of MgATP at 3 Å resolution, and of MnATP at 2.5 Å resolution are sufficiently detailed for the conformation¹⁷ of the bound nucleotide to be determined. The base is in the *anti* conformation with respect to the sugar, ($\chi \sim 80^\circ$); the sugar pucker is C3' *endo*; and the conformation about the exocyclic C4'-C5' bond is *gauche*(-) (or *trans-gauche*). The triphosphate chain of ATP is in a somewhat coiled conformation and directed away from the adenine

Table 1 Statistics of the X-ray analysis

Phase set	No. of atoms	<i>R</i> *	Figure of merit
Isomorphous	—	—	0.46
Combined set 1	1969 (main chain)	0.56	0.54
Combined set 2	2472 (main chain + 200 side-chains)	0.45	0.62
Combined set 3	3016 (main chain + 400 side-chains)	0.32	0.73
No. of intensities measured for native set to 2.5 Å			
		32,900	
No. of unique reflections to 2.5 Å		13,000	
Merging <i>R</i> † for native set		0.04	

$$R^* = \frac{\sum (F_{\text{obs}} - F_{\text{calc}})}{\sum F_{\text{obs}}} \quad R^\dagger = \frac{\sum (I_{\text{obs}} - I_{\text{mean}})}{\sum I_{\text{obs}}}$$

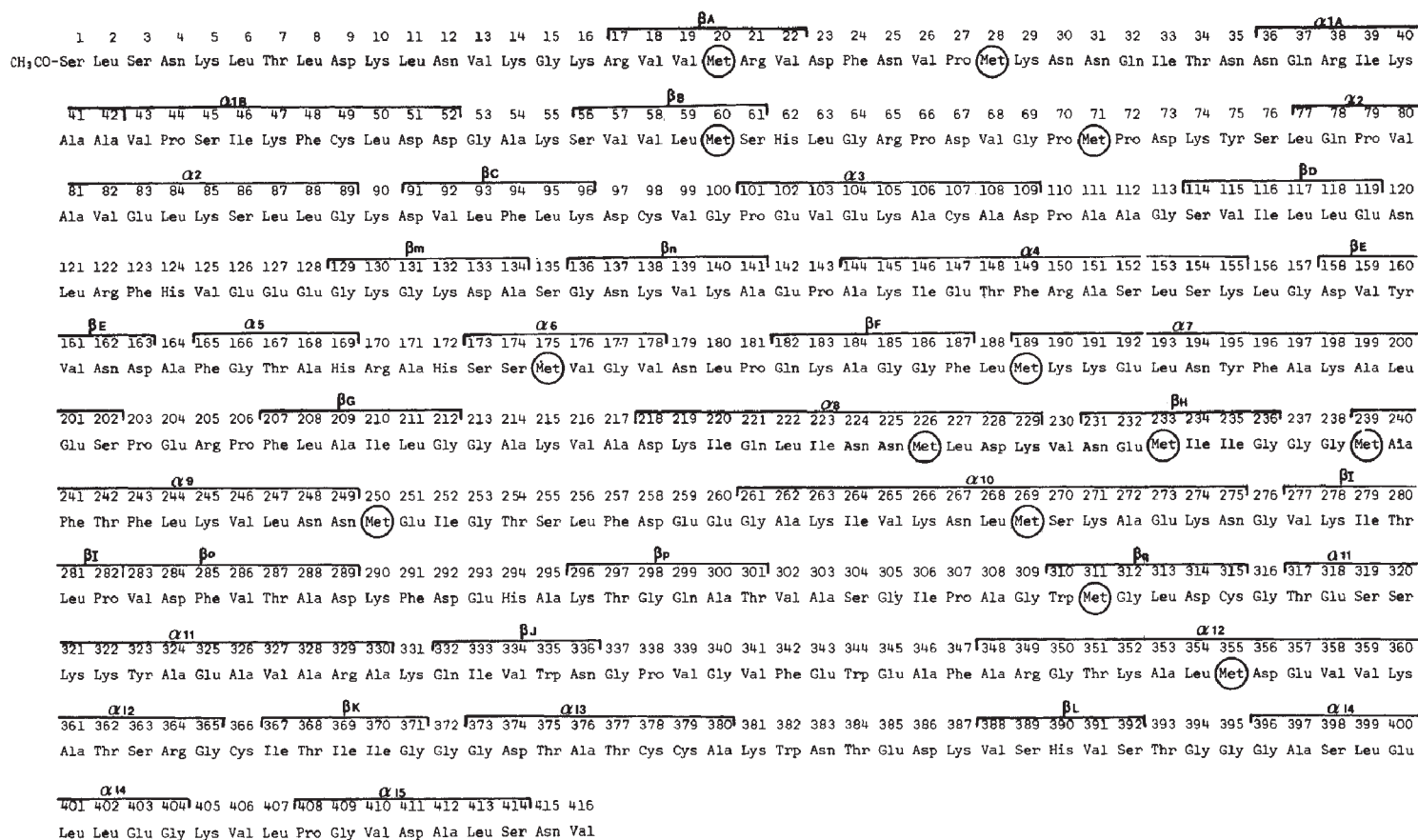


Fig. 1 Sequence of horse muscle phosphoglycerate kinase determined by ordering the 14 cyanogen bromide peptides on the X-ray map. The methionine residues that terminate each peptide are circled. The position and extent of the α -helices and β -strands are indicated, using the same nomenclature as in Fig. 2.

group. This conformation can be seen in Fig. 4; it is quite different from the conformation observed in crystals of ATP¹⁸. Bound ADP has the same conformation as bound ATP.

The major interactions between the enzyme and the nucleotides are shown in Fig. 4. The adenine ring is almost completely buried in a deep, narrow slot. The amino group is innermost and probably makes a hydrogen bond with main-chain carbonyl of Leu 313. The adenine slot is lined by the main-chain segments that immediately follow β -strands G, H and J: Gly 212, Gly 213 and Ala 214; Gly 236, Gly 237 and Gly 238; and Val 339, Gly 340 and Val 341, giving the site a similar character to the aromatic specificity site of chymotrypsin¹⁹. The ribose is located in a shallow depression above the pyrrolidine ring of Pro 338. Ribose binding involves the side chain of Glu 343 which appears to move and stiffen to form hydrogen bonds with probably both the 2'- and 3'-hydroxyls of the sugar. The α -phosphate group seems to make an ion-pair interaction with the charged ϵ -amino group of Lys 219. The β - and γ -phosphates of ATP are located about 5 Å from the amino-terminus of helix 13, but do not interact with any side-chains. Hol *et al.*²⁰ have recently shown that the phosphate moieties of bound NAD, FAD and other phosphate-containing ligands, are similarly positioned in relation to helices in other enzymes, and suggest that there is a favourable ionic interaction between the negatively charged phosphates and the positive dipole at the N-termini of helices. It seems reasonable to conclude therefore that helix 13 in PGK acts as a phosphate-binding helix as suggested by Hol *et al.*²⁰.

The binding of metal-ADP and -ATP complexes to PGK is accompanied by very similar small conformational changes in the vicinity of the binding site, and the only major difference between the interaction of the two nucleotides with the enzyme is in the location of the metal. Difference maps of the bound MgADP complex at 3 Å resolution in both sulphate and tartrate show a peak almost resolved from the main ADP density as shown in Fig. 3a. This peak coincides with the major peak in a

difference map at 6 Å resolution between MnADP and MgADP, and can therefore be reasonably ascribed to the metal ion. It is located about 4 Å from both the α - and β -phosphates, and close to the carboxylate of Asp 374 as shown in Fig. 4. In contrast none of the difference maps of metal-ATP complexes shows a peak at this position (see Fig. 3b). Instead, difference maps of MnATP at 2.5 Å resolution and of MnATP-MgATP at 6 Å resolution show low peaks about 4 Å from the γ -phosphate. This evidence suggests that the metal may be associated with the γ -phosphate of ATP as shown in Fig. 4.

This X-ray picture of the nucleotide binding site on horse PGK is in excellent agreement with that produced for the yeast enzyme from the NMR studies of lanthanide ADP and ATP complexes²¹. The conformation of bound ATP revealed by both techniques is essentially similar, and the close cluster of three

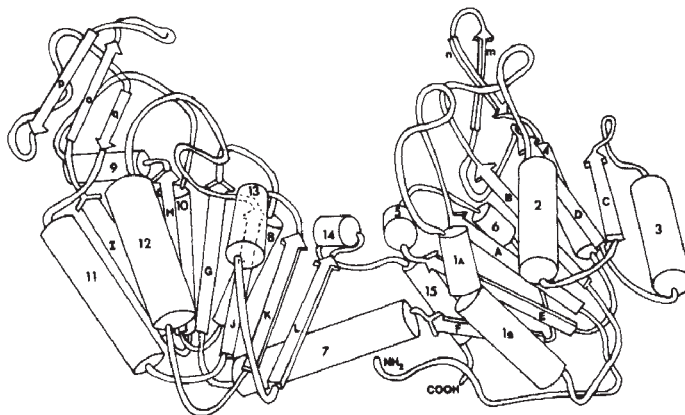


Fig. 2 A drawing of the structure of the native horse muscle phosphoglycerate kinase molecule. α -Helices are defined by cylinders and the β -strands by arrows which also denote their direction.

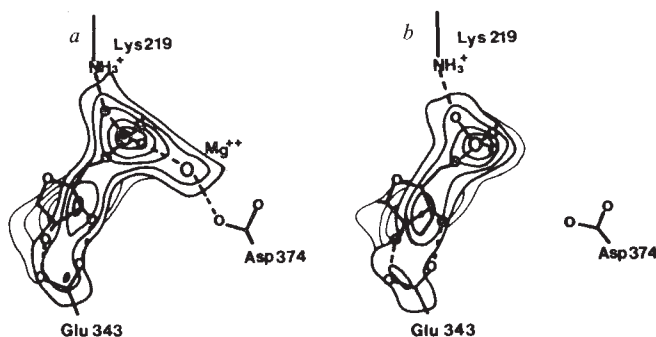


Fig. 3 Equivalent two sections through the difference maps of *a*, MnATP at 2.5 Å resolution and *b*, MgADP at 3.0 Å resolution, approximately in the plane of the ribose and α -phosphate (see Fig. 4). The adenine is below the plane to the left and the remaining phosphates above the plane to the right. The peak corresponding to the metal in ADP can be clearly seen, as can its absence from this position in ATP. Interchange of Mg^{2+} for Mn^{2+} has no effect on the metal position, nor on enzyme activity¹².

histidines located by NMR can be reasonably identified with the equally close cluster containing His 62, His 169 and His 172 shown in Fig. 4 by their similar position relative to the nucleotides.

In contrast to PGK crystals in sulphate, which do not interact with 3-phosphoglycerate even at high concentrations^{2,3}, the same crystals in tartrate exhibit large changes to their diffraction patterns at quite low concentrations of this substrate. However, difference maps calculated at 6 Å resolution reveal that the intensity changes are almost entirely accounted for by conformational changes that occur throughout the enzyme and most probably correspond to a rotation of the two domains relative to one another. Unfortunately it is not possible amongst the numerous peaks corresponding to the conformational change to identify any single peak as representing the bound substrate. A difference map calculated at 6 Å resolution of the ATP-3-phosphoglycerate ternary complex is similar to the sum of the difference maps of the two equivalent binary complexes.

Possible mode of action

It would be expected that location and definition of the binding site for one substrate in a two-substrate enzyme such as PGK

would lead to the identification, at least in general terms, of the binding site of the second substrate, and the catalytic site. This is not so on either count for PGK, as an examination of Fig. 4 shows. ATP is bound with its γ -phosphate, which is transferred by the enzyme, in a region of the molecule almost devoid of functional groups, and almost completely exposed to the solvent. The amino acids closest to the γ -phosphate include two triple glycine sequences, residues 371–373 and 394–396, and Leu 399. The nearest functional groups, Arg 38, His 169, Lys 215 and Glu 403 are about 10 Å distant. In this environment it is very difficult to discover any plausible binding site for 3-phosphoglycerate sufficiently close to the ATP for direct transfer of the phosphoryl group to occur; or any functional groups close enough to the γ -phosphate of the ATP to catalyse the transfer; or finally, how water could be excluded from the vicinity of the γ -phosphate during the catalytic reaction.

There seem to be three possible explanations of this apparent paradox: (1) the observed ATP does not represent the phosphorylating nucleotide; (2) the observed domain geometry of the enzyme is an artefact of crystallisation; (3) the phosphoglycerate substrates bind at a site distant from the nucleotide site. Although in relation to the first possibility the presence of more than one substrate binding site has been proposed to explain the nonlinear kinetics of homologous yeast²³, and human erythrocyte²⁴ enzymes (which may have a different origin as discussed later), the independent X-ray studies of the horse and yeast enzymes⁵, and NMR studies on the yeast enzyme²¹ all reveal the same single binding site. The second possibility seems highly unlikely because yeast PGK, the crystal packing of which is quite different to the horse enzyme, exhibits the same disposition of domains⁶. The direct test of the third possibility, by the X-ray examination of complexes of the enzyme with phosphoglycerate, was inconclusive because the induction of extensive conformational changes obscured the binding site.

In the present absence of experimental evidence, we have examined the molecular structure for plausible phosphoglycerate binding sites. We have used the following criteria: (1) as at physiological pH, 3-phosphoglycerate carries three negative charges, and the more strongly bound 1,3-diphosphoglycerate four negative charges, it seems reasonable to suppose the presence of a cluster of basic residues in their binding site; (2) because X-ray analysis of enzymes has shown that substrates are almost always located in surface depressions, a cavity capable of accommodating the relatively small phosphoglycerates was

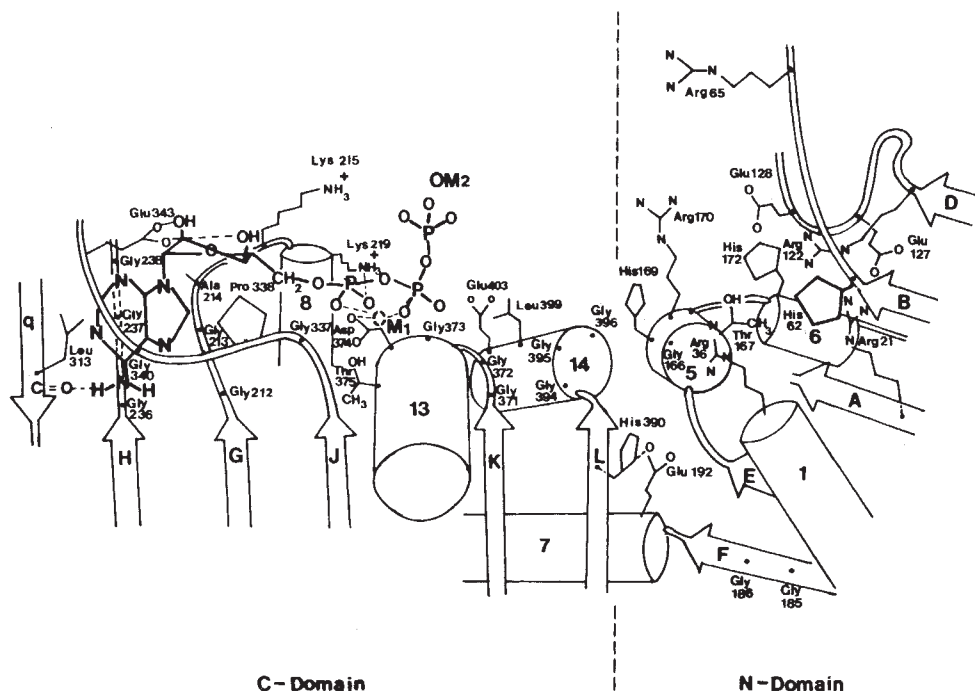


Fig. 4 A schematic drawing, approximately to scale, of the probable active site of phosphoglycerate kinase, with ATP bound. Bound ADP takes up the same position and conformation. The metal position in the ADP complex is shown by the broken circle (M_1), and that of the ATP by the full circle (M_2). This is an equivalent view to Fig. 2.

expected; (3) because there seems to be no catalytic residues near the γ -phosphate of the ATP, functional residues that could contribute to the catalysis may be located near the phosphoglycerate site. Using these criteria we found only one possible candidate for the binding site. This is shown in Fig. 4.

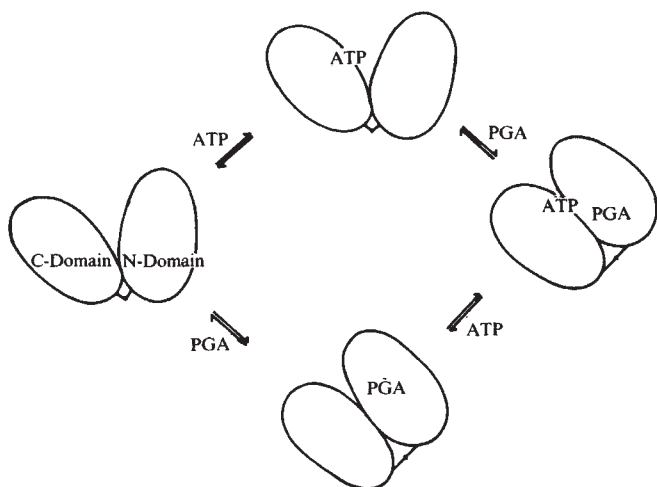


Fig. 5 Schematic drawing of the probable conformational changes in the substrate complexes of phosphoglycerate kinase suggested by the X-ray studies.

This site is located in the face of the N-terminal domain that opposes the ATP site on the C-terminal domain. The small cavity is located between the β -strands A and B and helix 6. Located within it and around it are five arginines, residues 21, 38, 65, 122 and 170; three histidines, residues 62, 169 and 172; two glutamates, residues 127 and 128; and aspartate, 23. Of these Arg 21 and Glu 127 are almost buried inside the cavity. This small region of the molecule contains half the arginine and histidine residues in the whole molecule. Note that in the NMR 'map' of the active site of yeast PGK²¹, the phosphoglycerate substrate is located further away from the ATP than the cluster of three histidines, which if identified with His 62, 169 and 172 of the horse enzyme, would place it in or near the cavity. If we assume that the observed structural homology between the yeast and horse enzymes extends to residues involved in substrate binding, the observation that at least one arginine takes part in the binding of 3-phosphoglycerate in the yeast enzyme²⁶ is also consistent with phosphoglycerate binding in the cavity.

If our tentative identification of the cavity with the phosphoglycerate site is correct, there is another problem to consider: as located on the native enzyme there must be a gap of about 10 Å between the γ -phosphate of the ATP and the carboxyl group of 3-phosphoglycerate that we presume must be closed when the phosphoryl group is catalytically transferred. Examination of the structure of the PGK molecule suggests that although each domain has a highly organised and apparently stable globular structure, the domain interface is small and not crossed by any of the secondary structures, suggesting that the enzyme is capable of a 'hinge-bending' conformational change. Consideration of Figs 2 and 4 shows that a rotation of 10–20° about a hinge located in or near the connection between β -strand F and helix 7 would be sufficient to bring the two domains, together with the ATP in its observed binding site, and the 3-phosphoglycerate in its proposed binding site, into contact, and at the same time to expel the solvent that lies between them. Residues 185 and 186 represent a Gly-Gly sequence at the C-terminus of β -strand F which could give the required degree of freedom at the appropriate position.

The 'hinge-bending' conformational change to PGK outlined above is illustrated in Fig. 5. We show the conformational change as occurring with the formation of phosphoglycerate

binary complexes, rather than with the formation of nucleotide binary complexes or ternary complexes, because our X-ray studies show that in the crystals in tartrate only small, local, conformational changes occur when ADP or ATP bind, whereas changes involving the whole molecule occur in the presence of 3-phosphoglycerate which are not increased in the ternary complex obtained by adding ATP. NMR studies of binary and ternary complexes of the yeast enzyme also show the same pattern of conformational change²¹, and studies on the rabbit muscle enzyme show that the reactivities of the seven sulphhydryl groups are affected to a greater extent by 3-phosphoglycerate than by ADP or ATP¹². One consequence of the conformational change is the possibility that it could mediate in allowing the enzymatic activity to be modulated by substrates and non-substrates, giving rise to the nonlinear kinetics, and activation and inhibition phenomena that have been observed with the human²³ and yeast enzymes^{24,25}, and which are otherwise difficult to reconcile with a monomeric enzyme. Although the 'hinge-bending' conformational change shown in Fig. 5 must be regarded as tentative until more information on the nature of binary and ternary enzyme-substrate complexes is obtained, it is consistent with most of the X-ray, NMR and chemical data discussed previously, some of which is difficult to understand in terms of a simple, monomeric enzyme.

Since preparing this paper we have become aware that Pickover *et al.*²⁷ have obtained direct evidence of a large conformational change in PGK from low angle X-ray studies of solutions of the yeast enzyme. They have found that the radius of gyration of the enzyme decreases by 1.09 Å on binding both ATP and 3-phosphoglycerate to form the ternary complex, but that binary complexes of these two substrates show much smaller decreases. Using the coordinates of the horse muscle enzyme they have interpreted the change in the radius of gyration in terms of a 'hinge-bending' conformational change of about 9–12° about a hinge located in the domain interface. Although the solution and crystal X-ray studies differ on whether the conformational change occurs on the formation of binary or ternary enzyme-substrate complexes, the proposed conformational changes are very similar: the illustration that Pickover *et al.*²⁷ provide of the enzyme after rotation of 12° about residue 187 (see Fig. 4) is extremely close to that which we have discussed above as being required to bring the observed ATP site and the proposed phosphoglycerate site together.

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Cloned fragments of the plasmid ColV,I-K94 specifying virulence and serum resistance

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A cloned BamHI-generated fragment of ColV,I-K94 increased the virulence of Escherichia coli, causing an approximately 100-fold reduction in LD₅₀ for chicks. A genetic determinant for resistance to the bactericidal effects of serum was mapped to a 5,300 base-pair sequence within the fragment. Neither colicin V nor immunity to colicin V affected the pathogenicity of E. coli for chicks.

STRAINS of *Escherichia coli* are an important cause of both diarrhoea and generalised infections in man and livestock¹. Enteropathogenic strains causing diarrhoea are usually confined to the alimentary tract and often harbour conjugative plasmids specifying enterotoxins² and K88 (or K89) antigen³ which enhance their pathogenicity. Smith^{4,5} found that plasmids could also enhance the virulence of invasive strains responsible for generalised infections. Most of the *E. coli* strains responsible for independent cases of generalised infections in calves, lambs or chicks produced colicin V, a plasmid-determined antibacterial protein. Elimination of the ColV plasmids from human, bovine, ovine and avian strains of several different serotypes invariably reduced their pathogenicity for experimental animals. Re-introduction of ColV plasmids into the strains by conjugation restored their pathogenicity to its original level. These results demonstrated that ColV plasmids increased the invasiveness of *E. coli*, but as Smith pointed out⁴, did not indicate whether colicin V itself or some other products specified by the ColV plasmids were responsible. The antibiotic effects of colicin V, like those of other colicins, are confined largely to enterobacteria; colicin V is not toxic for animals^{6,7}. However, the ecological significance of colicins is obscure (see ref. 8) and it remained possible that colicin V might increase the virulence of *E. coli*.

Isolation and characterisation of ColV derivatives

The genetic determinants for virulence and for increased survival in serum, which are probably identical as discussed below, were mapped by isolating deletion mutants and point mutants of ColV, I-K94 and by cloning fragments of the plasmid into the plasmid vector pBR322 (ref. 9) by *in vitro* recombination procedures. The ColV derivatives were isolated in *E. coli* K-12 strains and were then transferred into a recently isolated *E. coli* strain which was more suitable for testing the effects of plasmids on the pathogenicity of *E. coli* for experimental animals.

ColV, I-K94 is a conjugative (F-like) plasmid¹⁰⁻¹² specifying colicin I as well as colicin V¹¹. It was originally found in *E. coli* K94, a strain isolated from the stools of a patient suffering from a *Salmonella paratyphi* B infection (ref. 13 and P. Fredericq, personal communication). The positions of the genes specifying colicins and colicin immunity in ColV, I-K94 (pKH38) and in its derivative pKH46-1::γδ have been determined (D.L.D., M.M.B. and K.G.H., in preparation). The genetic and physical maps of ColV, I-K94 and the derivatives used here are shown in Fig. 1.

Most of the plasmids used in the pathogenicity tests were derived from pKH46-1::γδ, a *tra*⁻ deletion mutant of ColV, I-

K94 (pKH38) specifying colicins V and I. pKH46-1::γδ has an insert of the 5.7-kilobase γδ insertion sequence^{14,15} and was isolated after mobilisation of pKH46 by the F plasmid. The presence of the γδ sequence in pKH46-1::γδ and its derivatives increased the frequency with which these plasmids could be transferred by F mobilisation¹⁵ into the strain used for testing pathogenicity and serum resistance. This was an essential feature of these plasmids as the strain used for these tests (KH933, serotype 078:K80) was a poor recipient in matings with either derepressed F-like or I-like plasmids and could not be transformed (<1 transformant per 100 μg of supercoiled pKH46-1::γδ DNA using the procedure described by Cohen *et al.*¹⁶).

Spontaneous deletion mutants of pKH46-1::γδ which specified no colicin V or only reduced titres occurred at a frequency of approximately 0.1%. The deletions in the two mutants used here had one end in common, namely the end of one of the 2.1-kilobase inverted repeats in pKH46-1::γδ (Fig. 1). The positions of the deletions were determined by electrophoresis of fragments generated by restriction endonucleases, as described in Fig. 1 legend, and from heteroduplexes formed between the deletion mutants and derivatives of pKH46-1::γδ which had insertions of the transposon Tn5 (ref. 17) which specifies kanamycin resistance. The positions of Tn5 in these plasmids are shown in Fig. 1. Plasmid

Table 1 Colicin synthesis and immunity specified by derivatives of ColV, I-K94

Strain	Plasmid	Colicin titres		Colicin immunity	
		V	I	V	I
KH961	pKH46-1::γδ	64	512	+	+
KH974	pKH46-1::γδ Δ (7.4–25.6 kilobases)	<1*	512	+	+
KH982	pKH46-1::γδ Δ (7.4–29.3 kilobases)	—	256	—	+
KH962	pKH46-1::γδ <i>cia</i> -1	32	1	+	+
KH1020	pKH46-1::γδ 19.9kb::Tn5	64	256	+	+
KH1022	pKH46-1::γδ 19.9kb::Tn5 <i>cva</i> -1	<1	256	+	+
KH1069	pBH11	32	—	+	—
KH1070	pBH14	—	—	—	—
KH1071	pBR322: <i>Bam</i> HI-A	—	4096	—	+
KH1072	pBR322: <i>Bam</i> HI-C	<1*	—	+	—
KH1073	pBR322: <i>Hind</i> III-B	64	—	+	—
KH1074	pBR322: <i>Eco</i> RI-A	—	2048	—	+
KH932	ColV-B188	<1*	—	+	—
KH933	None	—	—	—	—

The host strain for all plasmids was KH933 (serotype 078:K80). For colicin titrations, broth cultures grown to $A_{600} = 0.8$ were disrupted by sonication for 35 s (ref. 24). Serial twofold dilutions of the sonicates were made and 0.02-ml volumes of each dilution were dropped onto the surface of 25-ml nutrient agar plates (Oxoid blood agar base no. 2) containing streptomycin (200 μg ml⁻¹) which had been overlaid with 3 ml of nutrient agar containing colicin-sensitive bacteria at a concentration of 2.5×10^6 cells ml⁻¹. Colicin I was assayed on plates overlaid with P1235 (ref. 37), a colicin V-tolerant mutant. Colicin V was assayed on plates overlaid with strain KH960, a colicin I-tolerant mutant of AB1157. Colicin titres are expressed as the highest dilution which inhibited growth of indicator bacteria. Colicin immunity was determined by streaking a loopful of broth culture (in exponential phase at 2×10^8 cells ml⁻¹) across a streak of colicin-producing bacteria (KH982 or KH1069) which had been grown for 24 h on nutrient agar, chloroformed and then overlaid with 3 ml of nutrient agar. Growth of colicin-immune bacteria (labelled +) was not inhibited over the streak. — Indicates absence of genetic determinant. Immunity to colicin I was tested using *E. coli* K-12 (W3110) as a host strain since KH933 is resistant to colicin I.

* These strains produced no detectable colicin V in broth cultures, but 48-h colonies on nutrient agar plates formed small inhibition zones when chloroformed and overlaid with strain KH960.

† Growth was partially inhibited by colicin V.

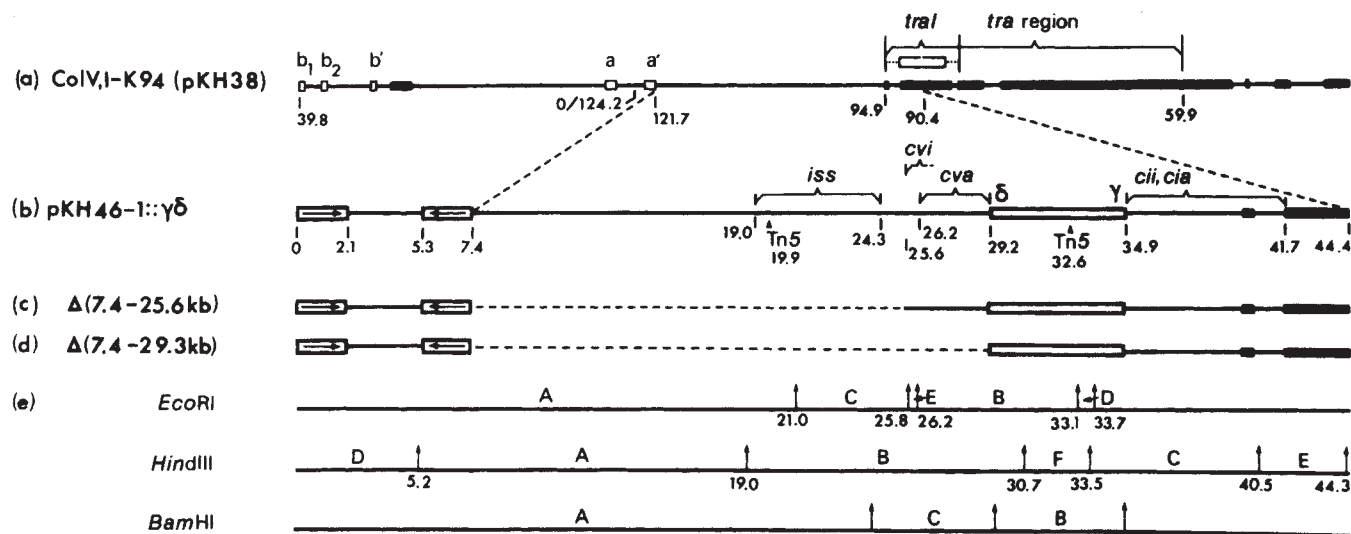


Fig. 1 Physical and genetic maps of ColV, I-K94 and its derivatives. *a*, ColV, I-K94 (pKH38). The positions of inverted repeat sequences (shown as open boxes) and of sequences homologous to the F plasmid in pKH38/F heteroduplexes (shown as thicker lines) were determined by electron microscopy of plasmid DNA (D.L.D., M.M.B. & K.G.H., in preparation). Coordinates in pKH38 (total length 124.2 kilobases) were defined, as described by Sharp *et al.*³², with reference to the 0.8-kilobase insertion/deletion loop at 87.1 F in pKH38/F heteroduplexes. ColV, I-K94 (pKH38) differs from the ColV, I-K94 molecules examined by Sharp *et al.*³² in the number and arrangement of 1.3-kilobase inverted repeat sequences and in not forming an insertion/deletion loop at 58.0 F in pKH38/F heteroduplexes. The *tra* region comprises the sequence which is homologous (as determined by heteroduplex analysis in the conditions described by Sharp *et al.*³²) to the F plasmid sequence 62.0 F–93.2 F which is essential for conjugation^{33,34}. Part of the ColV, I-K94 sequence which is homologous to the *traI* gene of the F plasmid occurs in pKH46-1::γδ as indicated in *b*. The *traI* gene of F maps within the homologous sequence indicated by dotted lines; the box represents the length of DNA sequence required to code for the protein specified by *traI* which has a molecular weight of 174,000 (ref. 34). *a,a'* Is an inverted repeat sequence of 1.3 ± 0.09 kilobases; *b₁, b₂, b'* are three homologous sequences of 0.8 ± 0.08 kilobases, two of which have the same orientation. *b*, pKH46-1::γδ is a deletion mutant of pKH38. The genes specifying colicin V (*cva*), immunity to colicin V (*cvi*), colicin I (*cia*), immunity to colicin I (*cii*) and increased survival in serum (*iss*) map within the regions indicated. The *cvi* gene maps to the left of *cva* as drawn, but the maximum limit of *cvi* to the right has not been determined. The positions of genes specifying colicins were determined from the analysis of cloned fragments and the relationship of pKH46-1::γδ to pKH38 was determined by heteroduplex analysis (D.L.D., M.M.B. & K.G.H. in preparation). The pKH46-1::γδ 2.7–5.3-kilobase sequence is homologous to the sequence between the 1.3-kilobase inverted repeat sequence (*a,a'*) in pKH38, but in pKH46-1::γδ it is flanked by a 2.1 ± 0.1 -kilobase inverted repeat sequence. The remaining 37 kilobases of pKH46-1::γδ (with the exception of the γδ insert) are homologous to the pKH38 90.4–121.7-kilobase sequence. pKH46-1::γδ coordinates are assigned from the end of one of the 2.1-kilobase inverted repeats. Copies of the Tn5 transposon were added to pKH46-1::γδ by mobilisation of the plasmid with *F'₆₁₁₄lac^r* (ref. 21) from a donor lysogenic for the bacteriophage λ _{B515B519X156C1857S7rex::Tn5} (ref. 17) to a *recA⁻* recipient in matings at 30 °C. Two recipients selected for expression of kanamycin resistance at 42 °C harboured plasmids with Tn5 insertions at the positions indicated. *c* and *d* are deletion mutants, pKH46-1::γδ Δ (7.4–25.6 kilobases) and pKH46-1::γδ Δ (7.4–29.3 kilobases). Restriction endonuclease sites within pKH46-1::γδ and its derivatives (*e*) were determined by electrophoresis of fragments generated by complete or partial digestion with restriction endonucleases (Boehringer) used either alone or in combination. Restriction endonuclease reactions were carried out for 15 min at 37 °C in 50-μl volumes containing approximately 0.5 μg DNA in the following buffers: 100 mM Tris-HCl (pH 7.5), 50 mM NaCl, 10 mM MgCl₂ (for *EcoRI*); 10 mM Tris-HCl (pH 7.6), 50 mM NaCl, 10 mM MgCl₂, 14 mM dithiothreitol (for *HindIII*); 6 mM Tris-HCl (pH 7.5), 20 mM KCl, 6 mM MgCl₂, 6 mM dithiothreitol (for *BamHI*). Fragments were analysed by electrophoresis through horizontal slab gels containing 0.8% (w/v) agarose and ethidium bromide (0.5 μg ml⁻¹) in Tris-borate buffer³⁵ at 60 V for 18 h at room temperature. Small fragments were analysed by polyacrylamide gel electrophoresis as described by Bolivar *et al.*⁹. Fragments of λ C1857S7 DNA generated by *EcoRI* and *HindIII* were included in gels as molecular weight standards³⁶. Fragments of pKH46-1::γδ were ligated to pBR322 (ref. 9) in a reaction mixture containing 0.066 mM ATP, 66 mM Tris-HCl (pH 7.6), 6.6 mM MgCl₂, 10 mM dithiothreitol, 1 unit T4 DNA ligase (Bethesda Research Laboratories) and 1 μg of restriction endonuclease-cleaved plasmid DNA in a total volume of 50 μl. After incubation for 4 h at 20 °C, recombinant plasmids were selected from the mixture by transformation¹⁶ and selection on nutrient agar plates containing ampicillin at a concentration of 120 μg ml⁻¹. Experiments involving recombinant plasmids were carried out in Class I conditions as defined by the Williams report (Cmdm 6600).

pKH46-1::γδ Δ (7.4–25.6 kilobases) specified greatly reduced titres of colicin V and immunity to colicin V (Table 1) and pKH46-1::γδ Δ (7.4–29.3 kilobases) specified no colicin V or immunity to colicin V. A point mutant of pKH46-1::γδ 19.9kb::Tn5 (pKH46-1::γδ containing the Tn5 transposon at the 19.9-kilobase position) which did not specify the synthesis of colicin V, was obtained by treating plasmid DNA at 37 °C for 24 h with 0.4 M hydroxylamine in the presence of 1 mM EDTA as described by Tessman¹⁸. A kanamycin-resistant transformant which did not specify colicin V synthesis (*Cva⁻*) was selected. The fragments of pKH46-1::γδ 19.9kb::Tn5 *cva*-1 generated by *EcoRI* and by *HindIII* were identical to those of pKH46-1::γδ 19.9kb::Tn5.

Fragments of pKH46-1::γδ obtained by digestion with restriction endonucleases *EcoRI*, *HindIII* or *BamHI* were cloned in the plasmid vector pBR322 (ref. 9) as described in Fig. 1 legend. Recombinant plasmids containing the following fragments were isolated: *EcoRI*-A, *BamHI*-A, *BamHI*-C and *HindIII*-B. In addition, part of the *HindIII*-B fragment, comprising the 22.6–30.7-kilobase sequence of pKH46-1::γδ, was cloned in pBR322 to form pBH11. pBH11 was obtained as a transformant from a *HindIII*-digested preparation of pKH46-1::γδ which was incubated with T4 DNA ligase in the presence of *HindIII*-digested pBR322. However, pBH11 was found to

have only one *HindIII* site although it specified both colicin V synthesis and ampicillin resistance. Analyses of restriction endonuclease digests of pBH11 and of the heteroduplexes pBH11/pBR322 and pBH11/pKH46-1::γδ 19.9kb::Tn5 established that the entire pBR322 sequence, but only part of the *HindIII*-B fragment, was present in pBH11. The orientation of the fragment in pBH11 was the same as in the plasmid in which the entire *HindIII*-B fragment had been cloned, that is, the sequence derived from the γδ insert (29.2–30.7 kilobases) was adjacent to the closely linked *HindIII* and *EcoRI* sites of pBR322. pBH14 was derived from pBH11 by ligating a 7.5-kilobase *EcoRI*-generated fragment of pBH11; it comprises the 22.6–25.8 kilobase sequence of pKH46-1::γδ and the pBR322 plasmid sequence with the exception of the 31-base pair sequence between the *EcoRI* and *HindIII* sites of pBR322.

Transfer of cloned fragments and ColV mutants to an invasive strain by mobilisation

To determine their effects on the pathogenicity of *E. coli* for chicks, ColV derivatives were transferred by conjugation into strain KH933, a Col⁻ derivative of *E. coli* B188. *E. coli* B188 was isolated from a calf suffering from a generalised infection and has the serotype 078:K80 (ref. 4), the most common

serotype among *E. coli* strains responsible for generalised infections in livestock^{1,4}. The ColV plasmid originally present in *E. coli* B188 was cured by treatment of the strain with acridine orange¹⁹ and its absence from the Col⁻ derivative, KH933, was confirmed by dye-buoyant density centrifugation²⁰. KH933 was chosen as the host strain for ColV derivatives so that the results of the pathogenicity tests could be compared with published data on the effects of other ColV plasmids^{4,5}. Furthermore, the effects of plasmids on a strain known to be a potential pathogen would be more significant than their effects on the pathogenicity of a strain such as *E. coli* K-12 which kills experimental animals only when given in huge doses.

Plasmid pKH46-1:: $\gamma\delta$ and its derivatives which also comprised a copy of the $\gamma\delta$ insertion sequence were transferred from auxotrophic *E. coli* K-12 donors using $F'_{h114lac^+}$ (ref. 21) as a mobilising plasmid in 8-h matings in broth at 30 °C. Recipients were selected on glucose-minimal salts agar²² containing kanamycin at a concentration of 25 $\mu\text{g ml}^{-1}$ or colicin V (produced by a lawn of ColV⁺ bacteria which was chloroformed after 18 h of growth, overlaid with 10 ml of agar and incubated for 4 h at 37 °C before being used for selection). F^- Col⁺ derivatives of the recipients were isolated from broth cultures which had been grown at 42 °C. Plasmid pKH46-1:: $\gamma\delta$ Δ (7.4–29.3 kilobases) was isolated as a spontaneous mutant from a KH933 strain harbouring pKH46-1:: $\gamma\delta$.

Derivatives of pBR322 were transferred to KH933 from *E. coli* K-12 donors which also harboured the I-like conjugative plasmid R144drd3 (ref. 23) and the non-conjugative plasmid ColK-K235 (ref. 24). ColK-K235 provided the *trans*-acting mobility determinant²⁵ which is essential for efficient mobilisation of pBR322 derivatives by R144drd3. Matings were interrupted after 5 min. Approximately 25% of recipients selected on plates containing ampicillin at a concentration of 120 $\mu\text{g ml}^{-1}$ were ColK⁻ and R⁻. Colicin titres specified by ColV derivatives in the host strain KH933 are listed in Table 1.

Table 2 Effect of ColV derivatives on the pathogenicity of *E. coli*

Strain	Plasmid	LD ₅₀ ($\pm$ s.e.m.) $\times 10^{-5}$
KH932	ColV-B188	2.4($\pm$ 2.4)
KH961	pKH46-1:: $\gamma\delta$	2.1($\pm$ 1.1)
KH962	pKH46-1:: $\gamma\delta$ <i>cia</i> -1	2.0($\pm$ 2.1)
KH1020	pKH46-1:: $\gamma\delta$ 19.9kb::Tn5	1.0($\pm$ 1.7)
KH1071	pBR322: <i>Bam</i> HI-A	1.8($\pm$ 1.8)
KH1022	pKH46-1:: $\gamma\delta$ 19.9kb::Tn5 <i>cva</i> -1	6.2($\pm$ 3.2)
KH974	pKH46-1:: $\gamma\delta$ Δ (7.4–25.6 kilobases)	220($\pm$ 170)
KH982	pKH46-1:: $\gamma\delta$ Δ (7.4–29.3 kilobases)	490($\pm$ 240)
KH1069	pBH11	380($\pm$ 200)
KH1070	pBH14	180($\pm$ 140)
KH1075	None	320($\pm$ 120)
KH933	None	190($\pm$ 180)

Pathogenicity was tested by injecting appropriate dilutions of broth cultures intramuscularly into 1-d-old chicks. For each strain, 14 chicks were injected at each of three or four concentrations of bacteria, ranging in 10-fold increments from 10^5 to 10^8 viable cells. Survival was recorded after 7 days. LD₅₀ values $\pm$ s.e.m. were calculated by probit analysis as described by Finney²⁶. KH1075 is a derivative of KH1069 which had spontaneously lost pBH11.

Pathogenicity of strains for chicks

Pathogenicity tests of strains for 1-d-old chicks (Table 2) showed that neither of the deletion mutants of pKH46-1:: $\gamma\delta$ nor pBH11, which specified high levels of colicin V, affected the pathogenicity of KH933. However, pBR322 linked to the *Bam*HI-generated fragment A (subsequently referred to as pBR322:*Bam*HI-A), which specified neither colicin V nor immunity to colicin V, increased the pathogenicity of KH933 as much as did pKH46-1:: $\gamma\delta$, pKH46-1:: $\gamma\delta$ 19.9kb::Tn5, pKH46-1:: $\gamma\delta$ *cia*-1 or ColV-B188. All these plasmids produced an approximately 100-fold decrease in the LD₅₀ of strain KH933 for 1-d-old chicks. The mutation in pKH46-1:: $\gamma\delta$ 19.9kb::Tn5 *cva*-1 which affected colicin V synthesis did not significantly affect the ability of the plasmid to increase the

virulence of *E. coli*. As pBR322:*Bam*HI-A increased pathogenicity but pKH46-1:: $\gamma\delta$ Δ (7.4–25.6 kilobases) did not, the virulence determinant can be mapped to the 7.4–24.3-kilobase sequence in pKH46-1:: $\gamma\delta$ and to the 104.8–121.7-kilobase sequence in ColV, I-K94 (pKH38). The slopes of the dose-response curves for all the strains tested were not significantly different; the ratios of the LD₅₀ values can therefore be used to compare the pathogenicity of strains.

Survival of strains in serum

Smith⁴ found that ColV⁺ strains survived better in a variety of animal sera than did their Col⁻ counterparts. Strains harbouring ColV derivatives were therefore examined to determine the position of the genetic determinant for increased survival in serum (*iss*). We used fresh rabbit serum, but Smith⁴ has previously shown that ColV plasmids also enhance survival in chicken serum. In addition to the plasmids used in the pathogenicity tests, the effects of the cloned fragments, *Hind*III-B, *Eco*RI-A and *Bam*HI-C, on the survival of KH933 in rabbit serum were examined. Of the 14 plasmids tested, the following did not affect the survival of KH933 in fresh serum: pKH46-1:: $\gamma\delta$ Δ (7.4–25.6 kilobases), pKH46-1:: $\gamma\delta$ Δ (7.4–29.3 kilobases), pBR322, pBH14, pBR322:*Eco*RI-A, pBR322:*Bam*HI-C. pBH11 caused a small but significant increase in survival (Fig. 2). The following plasmids greatly increased the survival of KH933 in serum: pKH46-1:: $\gamma\delta$, pKH46-1:: $\gamma\delta$ 19.9kb::Tn5, pKH46-1:: $\gamma\delta$ *cia*-1, pKH46-1:: $\gamma\delta$ 19.9kb::Tn5 *cva*-1, pBR322:*Hind*III-B, pBR322:*Bam*HI-A and ColV-B188. All these plasmids produced a similar increase in serum resistance, with the exception of pBR322:*Bam*HI-A, which was more effective than any of the others (Fig. 2). pBR322 is a multi-copy plasmid⁹; the high level of serum resistance specified by pBR322:*Bam*HI-A may therefore result from multiple copies of the *iss*⁺ determinant. The titres of colicin I specified by pBR322:*Bam*HI-A were eightfold greater than those specified by pKH46-1:: $\gamma\delta$ (Table 1). A strain harbouring both pBH14 and pKH46-1:: $\gamma\delta$ survived as well in serum as did a strain harbouring only pKH46-1:: $\gamma\delta$. All derivatives of KH933 grew equally well in rabbit serum in which complement had been inactivated by heating to 56 °C for 30 min.

The effects of ColV derivatives on the survival of bacteria in serum are therefore correlated with their effects on pathogenicity; only plasmids which greatly increased the survival of KH933 in serum enhanced the pathogenicity of the strain for chicks.

Both pBR322:*Hind*III-B and pBR322:*Bam*HI-A increased the survival of KH933 in serum, so the *iss*⁺ determinant maps within the 5.3-kilobase sequence at 19.0–24.3 kilobases in pKH46-1:: $\gamma\delta$. Within this region, neither the 19.0–21.0-kilobase sequence nor the 22.6–24.3-kilobase sequence encodes all the information required for expression of the *iss*⁺ determinant, as neither pBH11 (containing the 22.6–30.7 kilobase fragment) nor pBR322:*Eco*RI-A greatly increased survival in serum. In addition, introduction of the Tn5 transposon at the 19.9-kilobase position does not affect the serum resistance or the increased virulence conferred by pKH46-1:: $\gamma\delta$.

Seven of the ColV derivatives were also examined for their effects on the survival of *E. coli* K-12 in serum. All *E. coli* K-12 strains harbouring derivatives of pKH46-1:: $\gamma\delta$ were killed much more rapidly by fresh rabbit serum than were derivatives of KH933. However, plasmids which increased the pathogenicity and the serum resistance of KH933 also increased the survival of *E. coli* K-12 (strain W3110). The survival of *E. coli* K-12 strains was tested as described in Fig. 2 legend, except that bacteria were added to serum at an initial concentration of 10^7 ml^{-1} . All the following plasmids produced a similar increase in the survival of W3110 in fresh rabbit serum: pKH46-1:: $\gamma\delta$, pKH46-1:: $\gamma\delta$ 19.9kb::Tn5, pKH46-1:: $\gamma\delta$ 19.9kb::Tn5 *cva*-1. None of the following plasmids increased the survival of *E. coli* K-12 in serum: pKH46-1:: $\gamma\delta$ Δ (7.4–25.6 kilobases), pKH46-1:: $\gamma\delta$ Δ (7.4–29.3 kilobases), pBH11, pBH14. For example, the

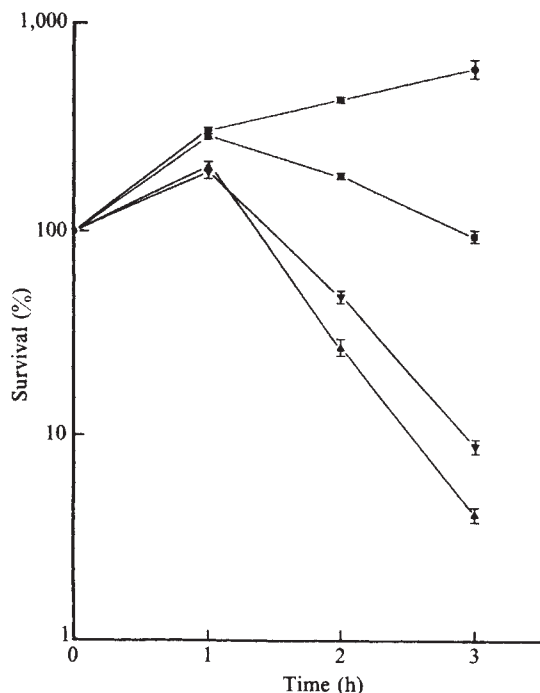


Fig. 2 Survival of bacteria in serum. Cells from exponentially growing broth cultures at $A_{600} = 0.4$ were collected by centrifugation and resuspended in an equal volume of 0.85% (w/v) NaCl. The suspension was diluted 100-fold in 0.85% (w/v) NaCl and then 10-fold in fresh rabbit serum. Rabbit serum was kept at -20°C after collection and was used within 18 h. All strains were tested simultaneously using the same batch of serum. The suspension of bacteria in serum was incubated at 37°C and viable counts were made at intervals by spreading 0.2-ml volumes of appropriate dilutions onto the surfaces of nutrient agar plates (Oxoid blood agar base no. 2). The value at each point is the mean $\pm$ s.e.m. of three independent determinations using the same batch of serum. Each of the three determinations was the mean of three spread plates. The strains are KH933 (▲), and KH933 harbouring the plasmids pBR322::BamHI-A (●), pKH46-1::γδ (■) and pBH11 (▼).

fractions of strain W3110 (pKH46-1::γδ) surviving in serum after 30 and 60 min were 6.4×10^{-2} and 2.9×10^{-2} . Comparable values for a W3110 strain which did not harbour a plasmid were 7×10^{-3} and 3.1×10^{-4} .

Implications of findings: close linkage of genes specifying colicin V and virulence

The correlation between the increased pathogenicity and the increased serum resistance specified by the ColV derivatives examined here strongly suggests that the primary reason for the increased virulence of bacteria harbouring ColV plasmids is that they are less sensitive to host defence mechanisms dependent on antibody and complement. The determinants for serum resistance and for colicin V are closely linked in ColV, I-K94; the *iss*⁺ determinant maps within the pKH46-1::γδ 19.0–24.3-kilobase sequence and the genes specifying colicin V and immunity to colicin V map within the 25.6–29.2-kilobase sequence (Fig. 1).

The presence of a ColV plasmid which increases virulence is not, of course, the only factor which is required to convert an otherwise benign strain of *E. coli* (such as *E. coli* K-12) into an invasive strain. Nonetheless, the high frequency of colicin V producers among *E. coli* strains responsible for recent cases of generalised infections in livestock⁴ suggests that it is an important factor. The occurrence of the virulence determinant on plasmids specifying colicin V is not confined to recently isolated plasmids from animal sources. The ColV, I-K94 plasmid used here was isolated from a patient in 1948. The first colicin to be investigated, by Gratia²⁶, was produced by a strain called *E. coli* V because of its virulence for rabbits and guinea pigs (the strain was isolated from the blood of an infected

rabbit), suggesting that the ColV-CA7 plasmid present in *E. coli* V also has the virulence determinant.

The frequent occurrence of the genes for colicin V and for virulence on the same plasmid, and their close linkage in ColV, I-K94, suggest that they may interact to their mutual selective advantage. The nature of any such interaction is, however, obscure, as is the ecological significance of colicins⁸. Several ColV plasmids increase the ability of *E. coli* strains to survive in the human alimentary tract^{4,5}, but this does not result from the bactericidal effects of colicin V⁵. It may be another effect of the *iss*⁺ determinant. The association between the genes specifying colicin V and virulence in ColV plasmids resembles the relationship between genes specifying raffinose utilisation and K88 antigen, in that almost all plasmids specifying K88 antigen, which aids colonisation of the small intestine by enteropathogenic strains, also specify raffinose utilisation, which is apparently unrelated to virulence²⁷. However, these two genetic determinants are not closely linked in at least one plasmid; in pRI8801 they are about 30 kilobases apart²⁸.

Whatever the reason for the relationship between the genes specifying colicin V and virulence on ColV plasmids, colicin V production may be a useful indicator of the presence of the virulence determinant in *E. coli* strains. It may be significant, for example, that colicin V is the type of colicin most frequently produced by *E. coli* strains responsible for urinary tract infections in hospitalised patients²⁹. Other reports³⁰ indicate that colicin V may be more commonly produced by strains responsible for a variety of human extra-intestinal infections than by strains isolated from the faeces of healthy people. However, the results reported here show that the virulence determinant of ColV, I-K94 is effective in the absence of genes specifying colicin V and immunity to colicin V. It may therefore be more widespread than is indicated by the frequency of colicin V production. Plasmids which do not specify colicin V but which increase the resistance of *E. coli* to the bactericidal effects of human serum have been described (see ref. 31). It is not known whether these plasmids also increase virulence.

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letters

Soft X-ray emission from the vicinity of the dwarf nova AY Lyrae

THE discovery of a low-intensity soft X-ray source in a region of the sky containing the dwarf nova AY Lyrae is reported here. Figure 1 shows how the source, designated H1839+37, appeared in the superposition of HEAO A2 scanning data that were taken on 9 October 1977 with the low-energy detector LED1. (A complete description of the A2 experiment on HEAO 1 is given by Rothschild *et al.*¹.) The source was detected only in the energy interval 0.18–0.43 keV. The peak in Fig. 1 represents an intensity of 0.014 ± 0.0027 counts $\text{cm}^{-2} \text{s}^{-1}$ (5σ). An upper limit to the temperature of H1839+37 was derived by comparing the ratio of the source counts in different energy bands. If a thermal bremsstrahlung spectrum is assumed, $T_{\text{BR}} \leq 5 \times 10^6$ K, whereas a black-body fit implies $T_{\text{BL}} \leq 1.4 \times 10^6$ K. A lower limit $T_{\text{BL}} \geq 1 \times 10^5$ K is set by the LED1 lower-level discriminator.

The region of the sky containing H1839+37 was scanned by HEAO 1 for several days. The light curve for the entire October 1977 observation is shown in Fig. 2a. Each point is the superposition of 1–5 individual scans made on a given day. For a half day, over which the spin axis of the satellite is held fixed, an estimate of the variability of H1839+37 can be made independently of knowing the exact source position, that is, without requiring corrections for the transmission of the LED1 collimator. Applying a χ^2 test to the five 9.5–10.0 scans for October (not shown here), the source is found to be constant at only the 10% confidence level ($\chi^2 = 7.68$ for 4 d.f.). The source is definitely variable on longer time scales because when it was observed 6 months later it was not detected, even in a superposition of 5.5 days of scanning data. The upper limit to the intensity for that observation (7–12 April 1978) is 0.005 counts $\text{cm}^{-2} \text{s}^{-1}$.

The centroid of the position error box for H1839+37 is at $\alpha = 18 \text{ h } 38 \text{ min } 58 \text{ s}$, $\delta = +37^\circ 52.4'$ (1950 epoch). The error box, shown in Fig. 3, excludes the bright star Vega (α Lyrae).

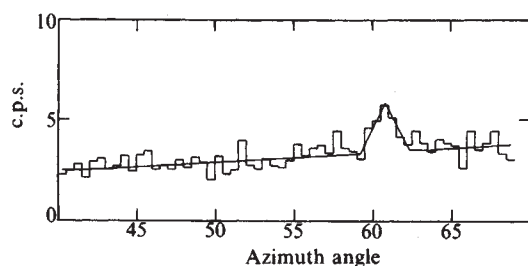


Fig. 1 Part of the data from a superposition of soft X-ray (0.18–0.43 keV) scans on 9 October 1977. The intensity of the source at scan angle 61° was determined by making a least squares fit (the smooth line) to the data. The fitting procedure took into account the triangular response of the collimator, which had a 1.5° field of view (FWHM) in the scan direction. The area of the detector was 174 cm^2 .

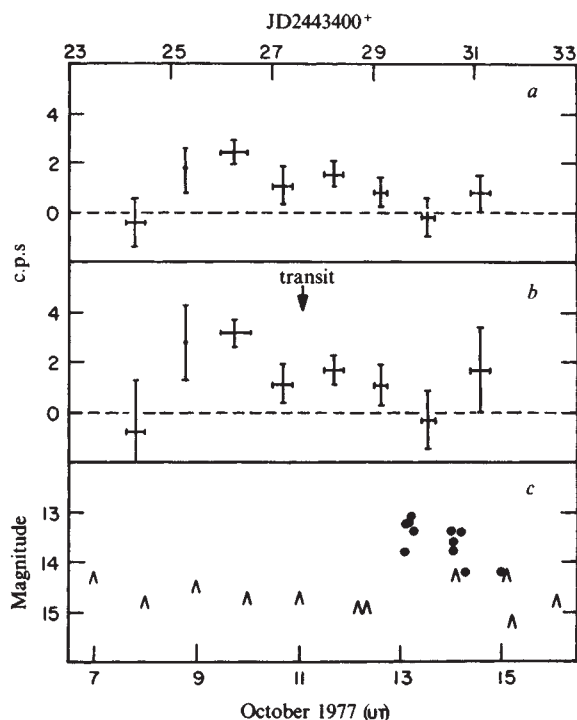


Fig. 2 a, The soft X-ray light curve of H1839+37 for October 1977. b, Same as (a), corrected for a source at the position of AY Lyr. c, The AAVSO visual light curve for AY Lyr covering the HEAO 1 observation.

Also shown is the slightly overlapping error box for 4U1852+37, a hard X-ray source with an intensity of 0.57 ± 0.15 Uhuru flux units (UFU) (ref. 2 and Jones, personal communication). The HEAO A2 upper limit for hard X-ray emission from either H1839+37 or 4U1852+37 is $1/3$ UFU (Swank, personal communication).

H1839+37 was discovered in a survey of ~ 130 cataclysmic variable stars³; it corresponds to the position of one of the members of this class—the dwarf nova AY Lyrae. Three other dwarf novae have been identified with soft X-ray sources: SS Cygni, EX Hydrae, and U Geminorum. This, plus the fact that a search in the H1839+37 error box has revealed no other promising candidates, make AY Lyr seem like a possible counterpart for H1839+37.

AY Lyr belongs to the subset of dwarf novae that have both normal outbursts and super outbursts⁴. Patterson⁴ reports that the quiescent visual brightness of the system is $V > 18$. At the time of the HEAO 1 sighting on 9 October 1977, AY Lyr was at $V > 14.8$ (Mattei, personal communication). Assuming a flat response across the LED1 bandwidth (0.18–0.43 keV), the 0.25 keV flux from a source at the position of AY Lyr is $\sim 1 \times 10^{-11} \text{ erg cm}^{-2} \text{s}^{-1}$. The ratio of the X-ray to optical flux for the other dwarf novae in quiescence is ≤ 1 (ref. 3). For AY Lyr, this ratio does not exceed unity only if the dwarf nova was as

bright as $V \sim 15$ during the HEAO 1 observation. In Fig. 2b the October 1977 light curve of H1839+37 has been corrected for a source at the position of AY Lyr, taking into account the transmission of the LED1 collimator. A comparison of the X-ray light curve with the AAVSO visual light curve for AY Lyr, which is shown in Fig. 2c, reveals that there was no increase in the X-ray activity during a short optical outburst of the dwarf nova⁵. There were only three LED1 scans during the October outburst; these occurred 0.4–0.6 d after the beginning of the outburst. A short visual outburst also occurred on 9 April 1978 when AY Lyr was again being scanned by HEAO 1 (ref. 5), but no X rays were detected from the object at that time either.

Part of the difficulty in interpreting this observation is in not having a good measurement of the X-ray spectrum of H1839+37. The dwarf novae detected as X-ray sources seem to have two very different spectral components: a medium or hard X-ray ($E > 0.5$ keV) component which fits a thermal bremsstrahlung model with line emission⁶, and a soft X-ray ($E < 0.5$ keV) component whose spectrum may fit any of several models³. The relative intensities of both components are correlated with the dwarf nova optical outburst. The soft component increases with increasing visual brightness, but the optically thin component is not uniquely correlated: in SS Cyg this component dramatically decreases during outburst⁷, while in U Gem the converse is observed⁸. In addition, the range of temperatures for the 'hard' component spans an order of magnitude: from ~ 20 keV for SS Cyg to ~ 5 keV for EX Hya and U Gem (refs 6 and 8). Therefore, with so few examples and such a wide range of behaviour, it is not difficult to envisage a behaviour pattern for H1839+37 which is consistent with the X-ray behaviour of dwarf novae.

For example, in AY Lyr variable accretion onto a white dwarf primary could account for X-ray flaring during quiescence. If the spectrum were optically thin, then absorption by increased in-falling material during outburst could produce a decrease in the bremsstrahlung X rays (an effect similar to that observed in SS Cyg during outburst). The bright soft X-ray excess observed in SS Cyg during outburst may not appear in AY Lyr because the boundary layer between the white dwarf and the compact star in AY Lyr may not get hot enough to radiate soft X rays. Differences in mass accretion rates during quiescence and outburst among all the dwarf novae may account for the range of observed behaviour.

Our intention has been simply to point out a correspondence in the positions of the soft X-ray emitter H1839+37 and the

dwarf nova AY Lyr, and to present all the pertinent data on both the X-ray and optical sources. Note that a search for a set of 130 randomly distributed error boxes ($\sim 0.6^\circ \times 6^\circ$ in size), such as the cataclysmic variable survey, would have about a 40% chance of producing a positional coincidence with one or more of the ~ 50 soft X-ray sources in the HEAO A2 Catalogue (in preparation). Thus there was a relatively high expectation of finding one fortuitous association of an X-ray source with a cataclysmic variable. If H1839+37 has a 'steady' emission even a factor of 10 below the HEAO 1 threshold, the X-ray telescope on the recently launched Einstein Observatory (HEAO 2) should be able to locate it accurately.

We thank Ms J. Mattei and the other members of the AAVSO for the October 1977 light curve of AY Lyr, Drs P. Szkody and K. Mason and the anonymous referee for their helpful criticisms, J. Patterson for his recent observations of AY Lyr, and J. Boyer for typing. This work was supported by NASA contract NAS 5-25049.

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Drift rates of Jupiter's S-bursts

THE average drift rates of Jupiter's S-bursts observed in the 21–23 MHz frequency range depend on the jovian declination of the Earth, D_E (ref. 1). The fact that the values obtained by other observers at higher and lower frequencies also seemed to agree with this observation was interpreted as either being coincidental or implying that the drift rates may not depend on frequency. Here I present new drift rate measurements at 21–23 MHz obtained in 1978, bringing the total number of individual measurements in 1974–78 to over 12,000.

The dynamic spectra of bursts were recorded on 16-mm film in real time with a 48-channel radio spectrograph. The S-spectra show a wide variety of forms². The simplest bursts appear as lines tilted in the orthogonal time-frequency plane. Samples of such events, from which drift rates are measured, are shown in Fig. 1. The samples illustrate the fact that the instantaneous bandwidth of bursts can vary considerably while the drift rate remains essentially the same.

All the simple bursts are observed to have a negative frequency drift. The rate varies from burst to burst and from group to group. The spread in rates within a storm is, as a rule, relatively large. The average drift rates do not seem to be related to the central meridian longitude (CML) of Jupiter or to Io's geocentric phase^{1,2}. The instantaneous bandwidths, however, tend to be larger at the high-longitude edges of region B. The S-bursts occur only in the Io-related regions B and C (refs 2, 3).

The mean drift rates for B-region storms observed in 1974–78 are plotted as a function of D_E in Fig. 2. As can be seen, the rates change from approximately -30 MHz s^{-1} to -20 MHz s^{-1} as D_E decreases from 3.3° to 0.5° . It is now obvious that the drift rates in the vicinity of -10 MHz s^{-1} which were obtained at other low values of D_E (refs 1, 4) must have been due to the lower observation frequency used, suggesting that the drift rates of S-bursts in region B are proportional to both D_E and frequency.

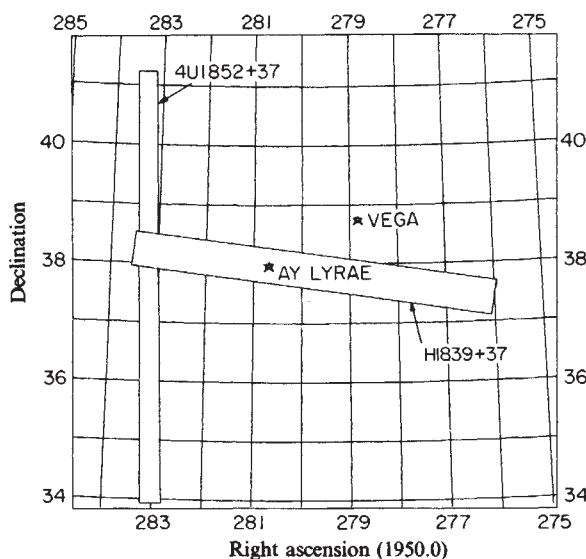


Fig. 3 H1839+37 error box (90% confidence), also showing the positions of Vega, the dwarf nova AY Lyr, and the error box for a 4U source.

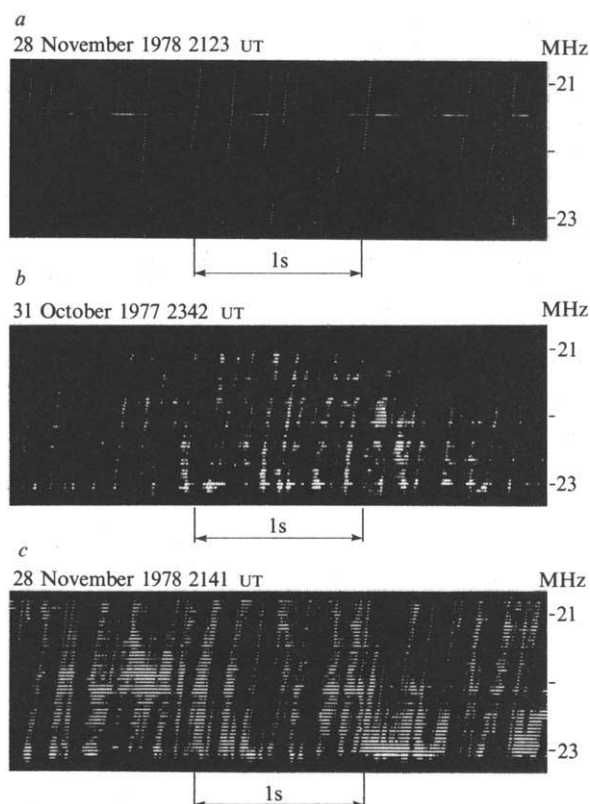


Fig. 1 Samples of high-resolution dynamic spectra of jovian S-bursts. Time increases from left to right. The instantaneous bandwidths of the bursts in *a* are 50 kHz or less, the bandwidths of certain bursts in *b* are a few hundred kHz, and those in *c* vary from 50 kHz to 1 MHz.

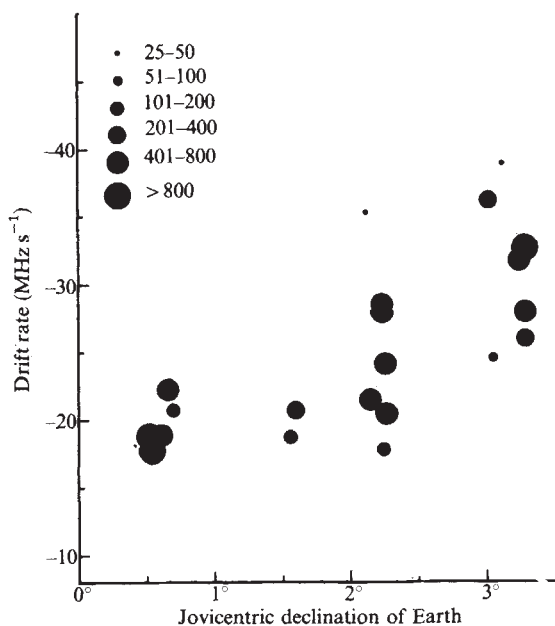


Fig. 2 Mean drift rates of S-bursts observed in storms of region B, plotted as a function of the jovicentric declination of the Earth. Each circle represents one storm, its size denoting the number of measurements.

If the mean drift rates of S-bursts in region C are plotted as a function of D_E , no clear dependence on D_E is evident. This is illustrated in Fig. 3, comprising storms observed in 1976 and 1978.

In a model often referred to, the sources of S-bursts move outwards from Jupiter along the magnetic field lines within the Io flux tube⁴⁻⁶. The frequency of emission is close to the local electron gyrofrequency, while the frequency drift is determined by the longitudinal velocity component of the source. The D_E -effect can be explained by assuming that the emission is sharply beamed so that a relatively small change in D_E may lead to a selection of bursts having different drift rates and different emission angles.

A similar selection effect should also occur due to the variations in the CML and the geocentric phase of Io. According to the Earth-Jupiter viewing geometry⁷, the viewing angle of an Io-threaded field line varies as a function of the CML and Io phase more than it does as a function of D_E . The storm-averaged drift rates, however, show no significant dependence on the CML or Io phase. Thus the evidence suggests that the explanation of the D_E -effect based on sharp beaming from upward-drifting electrons in the Io flux tube is not correct.

Another possible interpretation is that the variation of drift rates is not determined by a selection process but that the rates of all bursts vary together. In that case, a true physical relationship with the Sun may be involved, such as the direction of arrival of the solar wind, related to the jovicentric declination of the Sun, D_S (ref. 8). Since D_S never differs much from D_E , this possibility cannot be ruled out on the basis of the present series of observations covering less than half a cycle of D_E .

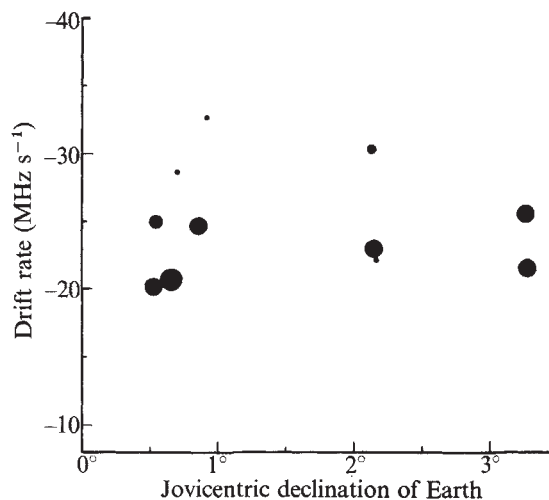


Fig. 3 Diagram as in Fig. 2, but for S-bursts observed in storms of region C.

One more possibility is that neither D_E nor D_S is the controlling factor, but that the solar activity is. If the storm-averaged drift rates are plotted as a function of the averaged sunspot number, an inverse relationship is displayed (not shown). This effect may be coincidental since the periods of D_E and of the solar activity are almost the same. Arguments relating to the relationships between D_E and D_S and the sunspot number have been presented previously in studies of the occurrence probability of L-burst and the longitude drift of emission regions^{8,9}. Many years of observation would probably be necessary to show whether the true correlation is with D_E (or D_S) or solar activity, or neither.

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Thermal conductivities of diamonds with absorption at 3.22 μm

MEASUREMENTS of the thermal conductivities of a hundred diamonds at temperatures between 320 and 450 K have been reported¹. These results were interpreted in terms of nitrogen impurity alone, although it was suggested that hydrogen might give an effect in other diamonds with absorption peaks at 3.22 μm wavelength. We report here measurements of the thermal conductivities of 24 natural diamonds with this IR absorption^{2,3}.

A variety of phonon scattering mechanisms determine the thermal conductivity of diamond as a function of temperature^{4–7}. Phonon scattering at crystal boundaries and large defects is dominant at low temperatures, whereas point defects are mainly important above room temperature. The conductivity of type IIA diamonds is extremely high over a wide temperature range. In the IR, these diamonds generally show only the fundamental lattice absorption (which is less than 1 cm^{-1} above 6.5 μm). Additional absorption bands in type I diamonds at 7.80 and 8.30–8.55 μm have been attributed to

Diamond no.	$k(320)$ ($\text{W cm}^{-1} \text{K}^{-1}$)	$k(450)$ ($\text{W cm}^{-1} \text{K}^{-1}$)	$p(3.22)$ (cm^{-1})	$a(7.3)$ (cm^{-1})	$a(7.80)$ (cm^{-1})	$a(\sim 8.4)$ (cm^{-1})
101	19.7	12.7	0.7	0.6	0.6	0.7
102	19.0	12.3	1.3	0.7	0.9	0.9
103	18.7	12.6	0.6	0.9	0.8	0.8
104	18.5	11.6	1.2	1.0	1.0	1.1
105	17.2	11.4	4.2	1.3	2.2	2.6
106	14.6	10.9	2.8	4.6	3.4	4.6
107	12.6	9.5	1.7	4.8	6.4	9.2
108	9.8	7.3	13.6	6.4	16.0	13.8
109	9.2	7.1	2.8	9.1	22	17.2
110	8.9	6.8	2.6	6.5	27	20
111	8.5	6.7	3.4	0.8	43	24
112	8.2	6.2	2.3	8.4	35	22
113	7.8	6.3	8.5	10.3	29	22
114	7.7	6.1	11.7	10.3	39	26
115	7.7	6.1	8.7	9.9	40	27
116	7.7	6.2	9.4	10.9	30	24
117	7.6	5.9	12.1	17.8	21	25
118	7.5	5.9	10.2	15.2	34	27
119	7.3	5.8	12.1	14.5	34	29
120	7.2	5.9	15.9	6.3	45	29
121	6.7	5.3	11.6	4.3	41	30
122	6.5	5.2	8.9	22	46	36
123	5.7	4.6	10.2	13.5	77	48
124	4.4	3.6	2.5	51	85	85

nitrogen⁸, which can be present in two forms in the diamond lattice^{9,10}. For the hundred diamonds of ref. 1, it was found that the maximum absorption in the range 8.30–8.55 μm can be used to predict the conductivity and it was concluded that nitrogen in concentrations higher than about 5×10^{-3} atm% gives detectable lowering of the conductivity.

The diamonds used in the present work were polished to rectangular bars free from cracks and inclusions at $\times 20$ magnification. Experimental details of the measurements of thermal conductivity and IR absorption were reported previously¹. Mean thermal conductivity values k at 320 and 450 K were derived from measurements of each diamond at these and intermediate temperatures by means of $k \propto T^{-s}$, where s is a constant for each diamond¹ (Table 1). The maximum absorption value in the range 8.30–8.55 μm is denoted as $a(\sim 8.4)$. The value $p(3.22)$ is the strength of a narrow absorption peak at this wavelength. It is defined as the peak absorption minus the fundamental lattice absorption (indicated by the trend of the absorption curve across the base of the peak). The 3.22- μm peak occurs in combination with one at 7.12 μm .

Diamonds 101–104 are type IIA diamonds with 3.22- μm peaks. Such diamonds seem to be rare. Their conductivities were found to be about as high as those of type IIA diamonds without 3.22- μm peaks. However, the 3.22- μm peaks of diamonds 101–104 are rather small and the diamonds with larger peaks also show absorptions due to nitrogen. Analysis of the absorptions of type I diamonds 105–124 gave no correlation between the amount or ratio of the two nitrogen forms and $p(3.22)$. Therefore, and in view of the existence of diamonds 101–104, the nitrogen impurity level seems to be independent of the value of $p(3.22)$. Diamond 124 has the lowest conductivity and a very high value of $a(\sim 8.4)$. From these values and ref. 1 it can be inferred that this diamond contains a very high concentration of nitrogen (0.5 ± 0.1 atm%). This has been confirmed by nuclear activation analysis¹¹.

The results for the 24 diamonds are compared with the mean results for diamonds without 3.22- μm peaks in Fig. 1. Values tend to lie below the line derived previously¹. An extra thermal resistivity

$$R(320) = \frac{1}{k(320)} - \frac{1}{k_L(320)} \quad (1)$$

has been calculated for each diamond. The subscript L refers to the line in Fig. 1 or, in the case of diamonds 101–104, to the value for type IIA diamonds. The results have been plotted

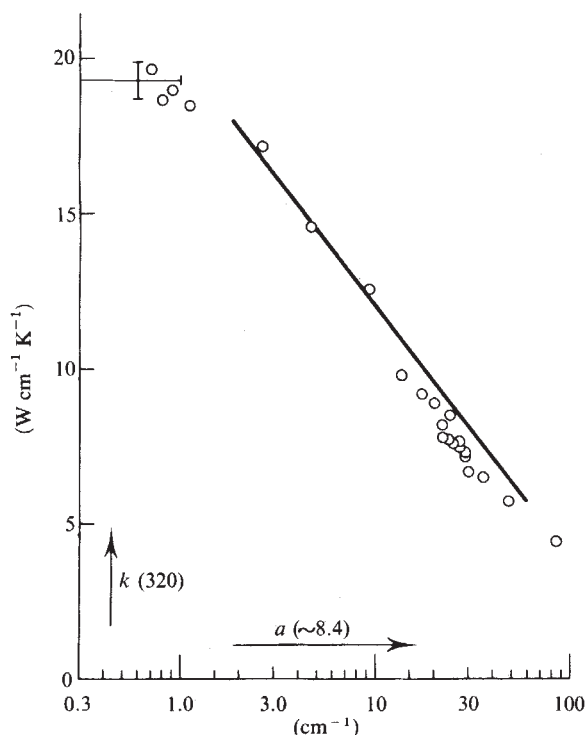


Fig. 1 Thermal conductivity at 320 K against maximum absorption at 8.30–8.55 μm . $\circ$, Results for diamonds with 3.22- μm peaks. The line represents the mean result for type I diamonds without 3.22- μm peaks. The mean result for type IIA diamonds without 3.22- μm peaks is shown with error bars representing 1 s.d. at upper left. Mean results from ref. 1 (Fig. 5).

against $p(3.22)$ in Fig. 2. It can be concluded that diamonds with large $3.22\text{-}\mu\text{m}$ peaks have a small but non-zero extra thermal resistivity. The same conclusion was reached from the data for 450 K.

This extra thermal resistivity probably arises from phonon scattering at hydrogen point defects in the diamond lattice. The presence of hydrogen impurity in some diamonds is well established, concentrations as high as 1 atm% having been reported by Sellschop *et al.*¹² and by Hudson and Tsong¹³. However, only one of Hudson and Tsong's diamonds showed the $3.22\text{-}\mu\text{m}$ absorption. Runciman and Carter³ attributed the 3.22 and $7.12\text{-}\mu\text{m}$ absorptions to stretching and bending vibrations of C—H bonds and estimated the hydrogen density at a

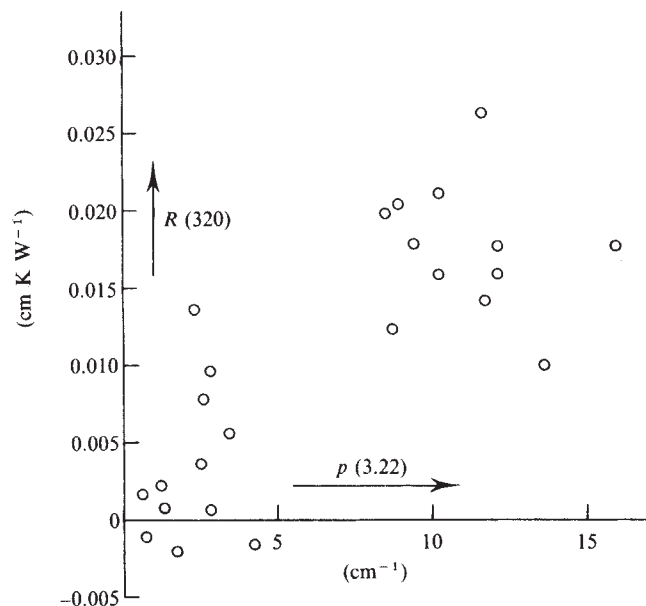


Fig. 2 Extra thermal resistivity at 320 K against the strength of the peak at $3.22\text{-}\mu\text{m}$. The accuracies of the ordinates are about $\pm 0.01\text{ cm K W}^{-1}$ and of the abscissae $\pm 0.3\text{ cm}^{-1}$.

much lower level (equivalent to 0.006 atm%), a result which they stated was confirmed by a vacuum fusion experiment. Chrenko *et al.*² also assigned these absorptions to C—H vibrations, but did not estimate the hydrogen concentration.

We believe that the larger hydrogen concentrations ($\sim 1\text{ atm}\%$) are most probably associated with relatively large impurity aggregates such as magma droplets¹⁴ or micro-inclusions¹⁵. These would not lower the thermal conductivity significantly above room temperature. Following Runciman and Carter³ it seems that the $3.22\text{-}\mu\text{m}$ absorption is probably associated with overall hydrogen concentrations some 2 or 3 orders of magnitude lower. These would then be the hydrogen point defect concentrations responsible for the extra thermal resistivity. This would be consistent with an extension to hydrogen defects of the analytical treatment of phonon scattering in diamond worked out by Turk and Klemens⁶ (as used for nitrogen by Berman and Martinez⁷). However, the assumptions in this extension of the theory are so great that we can draw no positive support from such consistency.

We conclude that there is a small extra thermal resistivity associated with the sharp $3.22\text{-}\mu\text{m}$ IR absorption in diamond. Both phenomena could be due to a small amount of hydrogen ($\leq 10^{-2}\text{ atm}\%$) in point defect form, but such causation has not been proved.

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Clouds and the long-term stability of the Earth's atmosphere and climate

CLOUDS dominate the albedo of the Earth and hence have a vital role in the global radiation balance. They are one of the most important physical properties of the atmosphere but the most difficult to parameterise. The forecasting of short-term climatological excursions (weather) requires numerical integration of complex dynamical models. However, over longer time periods it may be possible to include cloud cover without resorting to explicit atmospheric dynamics. Here we suggest that over evolutionary time periods (10^8 – 10^9 yr) the Earth's percentage cloud cover has remained approximately constant. This is in general agreement with present ideas about the stability of the Earth's evolution^{1–3}. Over medium-term climatological periods (10^4 – 10^7 yr) we have found that the position of large cloud masses may be directly related to the changing surface configuration, caused, for example, by continental drift. Global cloud cover fluctuates about a mean, which is near the present-day value and reinforces albedo changes caused by surface configuration; this could be highly significant for theories of climatic change.

The high reflectivity of clouds results in their dominance of the global albedo of the Earth even though they cover, on average, only about 50% of the surface area. Recently^{4,5} it has been demonstrated that the reflectivity in the visible wavelengths is more important than the complementary increase in IR opacity. Thus changing cloud amount cannot be ignored as had been suggested⁶, but must be considered as important in both long- and short-term climatic simulations. Cloud amount can be assumed to be a function of both the surface temperature and the available atmospheric water vapour. Contrary to some evolutionary models of the Earth's atmosphere⁷, we have found that the average global cloud amount has probably varied very little throughout the lifetime of the Earth.

Any long-term climatological model of the Earth must be concerned with calculation of the surface temperature, T_s , as we have observational data (from the geological record) for this parameter. It is generally agreed that throughout most of the planet's history^{2,8} the value of T_s has probably not varied significantly from that of the present day. Changes in the value of T_s may cause responses within the atmosphere–hydrosphere

Table 1 Albedo values, A , calculated for varying cloud cover

Percentage cloud cover	Albedo (%)
50 (present day)	30.1
40	24.6
60	35.7

system which could in turn perturb the global climate further. Some climate theories have developed the theme of temperature evolution via cloud changes⁷, although more recently^{1,8} the generally stable nature of the Earth's evolution and long-term climate has been emphasised. Here we examine the atmospheric response to changes in the global average surface temperature. As it seems that the albedo effect of cloud is of greater importance than the absorption in the IR wavelengths, it will be changes in the albedo via cloud changes which will lead to climate perturbations. Therefore we have used a computer model to calculate the Russel-Bond spherical albedo of the Earth. This model derives a value for the global albedo, A , by integrating the reflected intensity over all points of the Earth's surface, making allowance for variations in reflectivity and angle of incidence at each point. The model has been described in detail elsewhere⁹ and has been shown to give results consistent with measured values for A . The numerical integration includes the reflection due to clouds and is more satisfactory than calculations^{4,6} which simply derive the global albedo from

$$A = A_c \alpha_c + A_s (1 - \alpha_c) \quad (1)$$

where A_c and A_s are the average reflectivity values of cloud and surface and α_c the fractional cloud cover. Our model allows the importance and amount of both cloud and surface configuration to be estimated. The results have been used in two complementary modes below.

The major global sensitivity to changes in the surface temperature could be expected to come via the cloud response. It has recently been calculated¹⁰ that, contrary to the widely held view, increasing surface temperature leads to decreased cloud cover (as a percentage of the surface area covered). Sellers¹¹ has suggested that this decrease could be caused by the build-up of convective clouds rather than stratiform clouds (that is clouds with larger vertical extent but smaller horizontal area). Several complex general circulation models are producing this type of result^{6,12}. Roads¹⁰ suggests that the cloud amount will probably decrease by 1% per degree K increase in T_s . It is now possible to investigate the atmospheric response to changes in the surface temperature (within the ranges expected from the geological evidence).

We have considered very large changes in T_s (± 10 K) (compare the glacial/interglacial change of approximately 5 K) to illustrate the stability even in extreme conditions, but the results hold for smaller temperature changes also. If T_s were to be increased from its present value of 288 K to 298 K the resulting increased convection would lead to a decrease in the cloud amount of $\sim 10\%$ and hence to a lower value of A . The new value of A has been calculated from the computer model (see Table 1). The average atmospheric lapse rate can be estimated from the values of the surface temperature and the temperature at the tropopause. This tropopause temperature, T_{TOP} , is approximated by

$$T_{\text{TOP}} = 2^{-1/4} T_e \quad (2)$$

where T_e is the effective planetary temperature given by

$$\frac{S}{f} (1 - A) = \sigma T_e^4 \quad (3)$$

where σ is the Stefan-Boltzman constant, S the incident solar flux and f the planetary flux factor (which represents the ratio of the area of the planet emitting thermal radiation to the area intercepting solar radiation, and is a function of the planetary rotation rate and the absorption properties of the atmosphere^{8,13}). Thus for the present-day situation we have $T_s = 288$ K, $T_{\text{TOP}} = 214$ K and the resulting lapse rate of between 6 and 7 K km⁻¹ (in good agreement with measured values and more complex models¹⁴).

In the surface temperature increase thesis the new value of T_{TOP} is 218 K, and thus the lapse rate increases which, in turn, is likely (on a global scale) to enhance cloud formation (negative feedback). Similarly $T_s = 278$ K increases cloud, increases A to $\sim 35.7\%$ (see Table 1) and gives $T_{\text{TOP}} = 210$ K. The resulting lapse rate decrease would tend to inhibit further cloud build-up (of all types) and thus secure a return to a stable regime. Our contentions regarding the combined effect of changing the surface temperature and the atmospheric lapse rate are difficult to prove for the globally averaged situation we are discussing. However, similar arguments regarding the average atmospheric stability and the likelihood of cloud condensation have been considered for a number of planets by Goody and Walker¹⁵. Thus it appears that even large changes in T_s are unlikely to produce any catastrophic responses from the atmosphere.

The overall dynamic stability which is indicated by these results could, however, be disrupted by a secondary effect of changing cloud patterns via the possible variation in cloud positions. Obviously the cloud configuration on a global scale is a function of the general circulation. Within this framework the cloud cover may also be dependent on the predominant underlying surface type. Over very long time periods (10^6 – 10^8 yr) the relative positions of continents and oceans has changed greatly, and this may be an important factor for studies of the long-term temperature evolution. Henderson-Sellers and Meadows¹⁶ have shown that continental land mass movements are of importance for albedo changes and hence may be responsible for enhancing or stimulating glacial periods. This preliminary study excluded cloud cover perturbations. The data regarding cloud distribution are still extremely sparse, and any attempt to draw comparisons between 'continental' and 'oceanic' coverage patterns at this stage must be tentative. Studies have been carried out, however, to estimate the likelihood of cloud cover of surface features for surveillance from satellites¹⁷. This study describes cloud cover in terms of climatological regions. I¹⁸ have used this cloud data base to establish an objective method of cloud description using the beta probability distribution. From these studies it is possible to generate cloud probability maps (Fig. 1). These maps can show the areas most likely to be cloudy (or cloud free) over different time periods. In terms of the evolution of the global albedo certain features of Fig. 1 are particularly interesting.

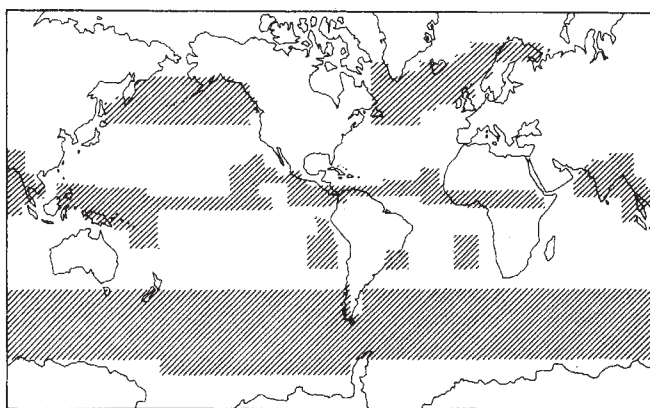


Fig. 1 Shaded regions have very high probabilities of cloud cover: July analysis (for details of analysis see ref. 18).

Table 2 Global albedo values, A , calculated for the two extreme continental configurations examined

Clouds	Albedo (%)	
	Configuration 1	Configuration 2
No clouds	6.8	12.1
50% Cloud cover		
present-day distribution	30.9	29.0
50% Cloud cover		
extent of ITCZ reduced	29.9	—
50% Cloud cover		
extent of ITCZ increased	—	31.1

Configuration 1 has all the continental mass close to the equator while configuration 2 has the continents within 40° of the poles.

Note that the high cloud cover zones appear to follow the surface features (open ocean areas) as well as the general atmospheric circulation. Hence we suggest that over very long time periods the consequences of changing cloud configuration need to be assessed, in spite of the above conclusion that total cloud cover may have changed little. We have used the computer program to generate albedo values for the Earth, including both latitudinal changes in the positions of the continents and the predominantly cloudy regions. We have retained 50% cloud cover in these calculations, whilst slightly perturbing the areas of dominant cloudiness (Table 2). Both the magnitude and direction of the change in A are significant.

Note that global albedo values only are presented here. The interactions between solar radiation and the terrestrial climate are extremely complex and depend on many factors. This complex system has recently been considerably trivialised¹⁹ by the suggestion that the current distribution of continental masses favours glaciation via the 'Milankovitch' orbital perturbation effect because of seasonality of temperatures (for example, the radiation distribution leading to cool northern summers and cold southern winters retaining and initiating ice cover respectively). This is contrary to the facts—climatological studies and more recent satellite data²⁰ immediately confirm that seasonal temperature change is greater in the Northern Hemisphere and that at present the Northern Hemisphere summer is warmer than the same season in the Southern Hemisphere. This is, of course, due to the global distribution of large oceanic areas and indicates that the 'Milankovitch' mechanism, if shown to be the cause of glacial epochs, operates despite the present global geography which opposes its effects in both hemispheres. Here we simply indicate the probable effect on the global radiation balance of cloud response to two extreme surface configurations.

The configurations chosen are: (1) all the land gathered closely around the equator; and (2) all the land areas within 40° of the poles. The albedo range in the absence of clouds is considerable¹⁶. However, imposition of present-day cloud reduces this difference to almost zero and reverses the ordering. We suggest from these studies that the global cloud cover could have responded to changes in the surface land/ocean configuration. We have therefore also calculated the likely global albedoes with a constant cloud cover percentage (50%) but with slight latitudinal changes in positioning of the predominantly cloudy areas. The change in A is considerable (7.2% for polar land masses, configuration 2). It is immediately clear that the postulated cloud changes re-establish the albedo difference between the two global configurations, the polar land mass case (configuration 2) still resulting in a higher value of A (although the difference between the two models is less than was previously anticipated¹⁶).

Our model calculations of the global albedo suggest two important conclusions. First, it seems that the average global cloud cover has remained approximately constant throughout the evolutionary history of the Earth's atmosphere. Second, a much smaller effect due to the reinforcement of surface forcing

of the albedo by perturbed cloud configuration is probably important in medium-term climatic change.

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Permo-Triassic and Jurassic ⁴⁰Ar-³⁹Ar ages from Greek ophiolites and associated rocks

PRECISE radiometric dating of ophiolites and associated rocks is usually very difficult as the rocks are often altered by ocean floor metamorphism or during tectonic emplacement onto the continental margin. Only age determinations on primary mineral phases can be considered to approximate their initial crystallisation and cooling ages. The K-Ar technique is generally used to date ophiolitic rocks, but even if primary mineral phases are analysed, there is always the uncertainty that the resulting age could have been partially reset by later thermal or tectonic events. The ⁴⁰Ar-³⁹Ar step heating method is able to detect and often to correct for any partial loss of argon, and thus overcome these problems. We have determined and report here ⁴⁰Ar-³⁹Ar age spectra for hornblende separates from two unaltered samples of igneous rocks that are interpreted as the initial igneous products of rifting in the Othris region of central Greece. A third hornblende has been analysed from amphibolites believed to have been formed during tectonic emplacement of the Pindos ophiolite (Fig. 1). The ages suggest that the initial rifting began in Othris in the Permo-Triassic (~248 Myr), while tectonic emplacement of the Pindos ophiolite occurred in the middle Jurassic time (~180 Myr).

The breakup of a continental area in what is now the Othris region began in early Mesozoic time¹. It is believed to be contemporaneous with the formation of picrites, wehrlites, dolerites, pillow lavas, tuffs and the deposition of associated cherts and dolomites assigned to the Agrilia Formation². The age of the Agrilia formation is not well constrained. In parts of Othris the underlying Gavriani Formation contains Upper Permian and Lower Triassic fossils, but the Gavriani/Agrilia boundary may be diachronous³. Previously determined K-Ar ages on altered whole rocks³ and amphibolite related to the emplacement of the ophiolites² were considered at best to be minimum ages of crystallisation (160, 185 Myr). ⁴⁰Ar-³⁹Ar analyses of primary hornblende from a wehrlite and a dacite in the igneous sequence have been obtained (Tables 1 and 2). Age spectra and associated Ca/K ratios are given in Fig. 2a and b.

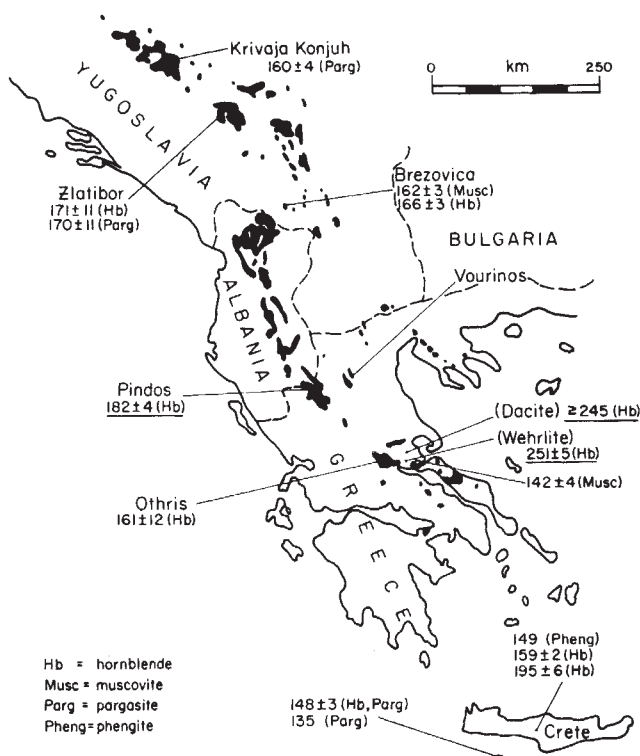


Fig. 1 Published K-Ar ages with new ^{40}Ar - ^{39}Ar data (underlined) on ophiolites and associated rocks in Yugoslavia, Greece and Crete^{2,13-15}. Decay constants recommended in ref. 16 used for all data, with time scale in ref. 6.

Details of analytical procedure will be given elsewhere, but duplicate analyses indicate the precision of the data.

The wehrlite hornblende spectrum is flat (Fig. 2a) and indicates a crystallisation age of 251 ± 5 Myr. In contrast, the dacite hornblende spectrum shows increasing age with increased

Table 1 Sample locations and characteristics

Sample*	Locality	Description and probable significance
G-16R 108185	~2 km west of Andinita, Othris Mountains	Wehrlite: Olivine (Fo84.5) poikilitically enclosed by augite and minor brown hornblende. Part of a gabbro-peridotite cumulate sequence related to picrites. Inception of rifting and igneous activity.
G-18 108201	~3 km south-east of Dhomokos, Othris Mountains	Dacite: Phenocrysts of brown hornblende, biotite, albitised plagioclase showing relict zoning and minor quartz. The groundmass has recrystallised to fine-grained albite K-feldspar. Isolated outcrop in plain. Felsic member of the Triassic volcanic suite associated with rifting.
B76-24 123656	Avominsta valley, Perivoli	Amphibolite: Green hornblende showing strong lineation plus plagioclase. Associated with garnet-mica schists. Probably continental margin material metamorphosed by ophiolite emplacement.

* Six-figure numbers from Harker catalogue, Department of Mineralogy and Petrology, Cambridge.

outgassing of ^{39}Ar (Fig. 2b). This age spectrum approximates the diffusion loss pattern predicted by Turner⁴ for later reheating and observed by Dallmeyer⁵ in hornblendes in a remetamorphosed terrane. If it is assumed that the spectrum is the result of diffusive loss of the argon, then the crystallisation of the hornblende is approximated by the highest temperature step and indicates an age of 245 ± 5 Myr or greater, similar to the wehrlite age. The minimum age observed in the low temperature steps suggests that the reheating took place at 150 Myr or later.

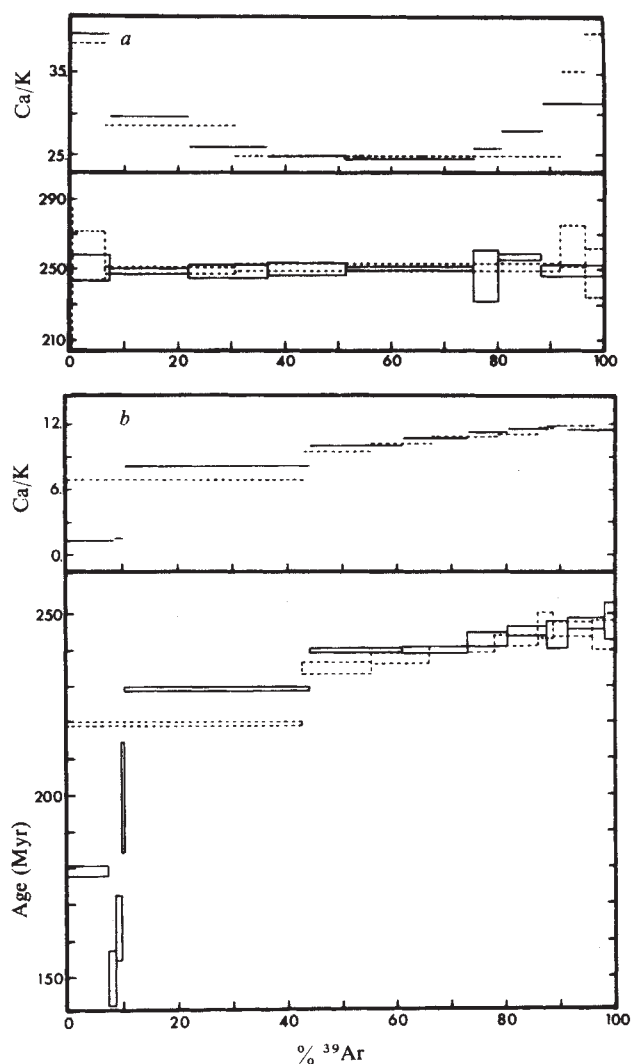


Fig. 2 a, Age spectra for duplicate analyses of G-16 wehrlite hornblende. Integrated age is 251 ± 5 Myr; 0.26% K. Error boxes (2σ) in all figures are analytical only and do not include uncertainties in the irradiation parameter ($\sim 1\%$). Errors on sample ages in the test and integrated ages do include this uncertainty. b, Age spectra for duplicate analyses of G-18 dacite hornblende. Integrated age is 231 ± 4 Myr; 0.80% K.

These two ages of ~ 248 Myr (near the Permo-Triassic boundary⁶) are the oldest ages so far determined on igneous rocks associated with initial rifting in central Greece. The ocean floor generated next to the continental margin cannot be older than these rocks, but it may be younger. Using the Red Sea as an analogy, Hynes¹ suggested that the initial igneous activity associated with rifting in the Othris region might precede truly oceanic rifting by as much as 40 Myr because of a slow rifting rate. However, Nisbet and Price⁷, on examining the cherts forming the upper member of the Agrilia Formation also

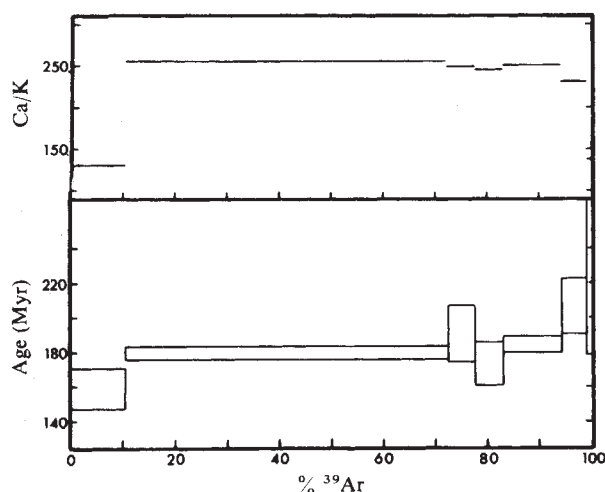


Fig. 3 Age spectra for B76-24 amphibolite hornblende. Integrated age is 181 ± 4 Myr. Duplicate total fusion ages are 186 ± 6 Myr and 188 ± 6 Myr; 0.036% K.

suggested a slow rate of rifting ($\sim 1 \text{ cm yr}^{-1}$), but they believed these cherts could have formed in an oceanic environment after only 7 Myr of rifting, resulting in an ocean basin 150 km wide. Thus a well-developed oceanic environment could have grown in a relatively short time period and the ocean floor could have an age indistinguishable within the error limits from the age of the early rifting volcanics (248 Myr). At present there are no biostratigraphic or radiometric data on the age of the ophiolites, but it is known that they were tectonically emplaced in Upper Jurassic or Lower Cretaceous time, about 135–145 Myr ago^{2,8}. Most workers agree that not only the Othris ophiolites, but the Vourinos and Pindos ophiolites were probably emplaced at this time^{9,10}, as well as some of the Yugoslavian examples¹¹.

Amphibolites and other metamorphic rocks developed at the base of ophiolites are currently interpreted as having been created by the tectonic emplacement of a relatively thick and probably hot sheet of ophiolite over continental margin sequences¹². Previously determined K–Ar ages on a hornblende separate and a whole-rock amphibolite from the rocks at the base of the Othris ophiolite gave average ages of 161 Myr. Because of the large analytical error (± 12 Myr), the amphibolites did not appear significantly older than the emplacement age suggested by field evidence—about 135–145 Myr.

However, five K–Ar determinations on metamorphic rocks below three Yugoslavian ophiolites lying in the same zone as the Greek ophiolites (Fig. 1) also gave emplacement ages ranging from 160 to 171 Myr^{13,14}.

We have analysed a hornblende separate (Fig. 3) from an amphibolite at the base of the Pindos ophiolite in northern Greece (Tables 1 and 2). Though initially emplaced in Mesozoic time, later Cenozoic thrusting has moved the ophiolite sheet so that it now lies tectonically on Eocene 'flysch'. This latest phase of emplacement has not reset the earlier Jurassic emplacement ages obtained from the amphibolites. The age spectra for 85% of the gas release is flat within the limits of analytical error and indicates an age of 182 ± 4 Myr. The age clearly shows that the amphibolites have not been created by Cenozoic thrusting but are related to an earlier Jurassic period of emplacement. In Othris the ophiolite has not been moved again by Cenozoic thrusting, but the field evidence clearly indicates an Upper Jurassic or Lower Cretaceous emplacement. Thus the amphibolites in the Pindos and Othris ophiolites are significantly older than would be suspected from field evidence, but similar to radiometric emplacement ages reported from Othris and Yugoslavia^{2,13–15}. We reconcile these data by postulating that the ^{40}Ar – ^{39}Ar age represents the time when a hot ophiolite sheet over-rode sediments and/or basic igneous rocks on the lower continental slope, whereas the field evidence gives the age of final emplacement of the ophiolite onto a continental platform some 40 Myr later in the case of the Othris ophiolite and some 140 Myr later in the case of the Pindos ophiolite.

The most important conclusions from these data are that igneous activity associated with initial rifting in this western belt of ophiolites in Greece may date from the Permo–Triassic boundary, and that ophiolite emplacement began in the lower Jurassic. Whether emplacement was slow and steady or fast and episodic cannot be decided from the available information.

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Table 2 Mineral analyses

Mineral	G16 (average of 4)	G18 (crystals zoned)		B76-24 (average of 3)
		from	to	
SiO ₂	45.8	41.8	41.2	47.8
TiO ₂	4.8	4.1	3.3	0.38
Al ₂ O ₃	7.9	10.5	9.4	10.9
FeO*	6.2	15.3	23.0	7.5
MnO	ND	0.22	0.49	0.14 (1 only)
MgO	16.5	11.1	6.1	15.6
CaO	11.0	10.9	10.5	12.2
Na ₂ O	3.4	2.7	2.2	1.3
K ₂ O	0.28	0.86	1.2	0.04†
Cr ₂ O ₃	1.1	ND	ND	0.19
Total	97.0	97.5	97.4	96.1

Probe analyses by W. E. Cameron [G16, G18] and J. Spray [B76-24].

* All iron expressed as FeO.

† Subsequent ^{40}Ar – ^{39}Ar analysis.

ND = not determined.

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Geological significance of a Middle Cambrian fauna from Antarctica

A NEW collection of a Cambrian fauna, occurring as erratics in a Quaternary moraine near Mount Provender (80°23'S, 29°55'W), western Shackleton Range (Fig. 1, inset) is reported here. It includes inarticulate brachiopods and trilobites, together with the first hyolithids and primitive molluscs from the area. Because of the isolation of the Shackleton Range, data on this fauna have accumulated only slowly¹⁻⁴. It is important because it is located at a geographical extreme of the known extent of the Cambrian in Antarctica, and because it represents a contribution to our knowledge of world Cambrian faunas. Although the provenance of the fossiliferous erratics is problematical, it has important stratigraphical implications concerning the controversial age of the Blaiklock Glacier Group, for which ages as far apart as Cambro-Ordovician^{2,3} and Permian¹ have been considered.

Other Cambrian faunas from Antarctica (Fig. 1) occur in the Transantarctic Mountains and in the Ellsworth Mountains of Lesser Antarctica. Like those of the Shackleton Range, they usually consist of assemblages of brachiopods and trilobites with or without associated archaeocyathids. In some instances exclusively archaeocyathid faunas have been found. The faunas range in age from late Lower Cambrian to Upper Cambrian⁵⁻⁹, although associated acritarchs at one locality in northern Victoria Land (Fig. 1, locality 9) suggest a possible brachiopod fauna might be as old as latest Precambrian¹⁰.

The Shackleton Range fauna occurs in fissile shales and siltstones. With the exception of the inarticulate brachiopods, which have shell material preserved, the fauna is present as internal and external moulds of moderate relief. In general, the brachiopods are preserved as unbroken but separated valves, the trilobites as disarticulated fragments, and the hyolithids as isolated conches. There are, however, moulds of completely articulated trilobite exoskeletons and broken brachiopod shell debris. The primitive molluscs are known from a single bedding plane on a small slab of shale; they are entire but laterally flattened.

All the brachiopods are identifiable with the obolids previously described from the area³. Two forms can be distinguished, one with thin smooth shells and the other with thicker shells having rows of concentric pits on the internal surfaces. Whether these represent two species or variants of the same species is uncertain.

The primitive mollusc is similar to some forms of *Helcionella* but is probably closer to *Mellopegma*¹¹, a possible intermediate between the Monoplacophora and Rostroconcha. The specimens are small (the largest is 3.3 mm long), laterally compressed cap-shaped shells with strongly recurved apices and coarse concentric growth corrugations.

The trilobites include agnostids of *Triplagnostus* and *Peronopsis* types, and ptychoparids of *Ehmania*, *Lyraspis* and *Elrathina* types. As pointed out by Soloviev and Grikurov⁴ these indicate a Middle Cambrian age for the fauna. The mollusc, if correctly assigned to *Mellopegma*, is also of stratigraphical value because its only previously reported occurrence is in the early Middle Cambrian of Australia. However, Runnegar and Jell¹¹ argued that morphologically similar forms must have existed as early as the Tommotian (early Cambrian).

Specimens in our collection suggest that, although the brachiopods and trilobites sometimes occur in mixed assemblages, they lived largely in different conditions. In general, the brachiopods occur in the siltstones or fine sandstones, whereas the trilobites occur more frequently in the shales. This, and the occurrence of fragmented shell accumulations, suggest that the brachiopods inhabited a slightly higher energy environment, periodically subjected to current action and reworking³.

The trilobite faunas of the Heritage, Neptune and Argentina Ranges and Harold Byrd Mountains all occur in limestones, although associated Cambrian successions also contain clastic rocks. Together with occurrences of archaeocyathid limestones it seems that a belt of intermittent limestone deposition was present along the site of the Transantarctic Mountains in Cambrian times. By contrast, the trilobites of the Shackleton Range occur in non-calcareous shales and siltstones. In this respect they resemble the upper Middle Cambrian fauna of the Mariner Group, northern Victoria Land⁹, where trilobites are present in fissile mudstones attributed on sedimentological criteria to an open marine environment¹². However, all known Antarctic Cambrian trilobite faunas are dominated by non-agnostids, a feature ascribed by Palmer^{13,14} to faunas whose access to the open sea was restricted. Only the *Aphelaspis*-rich fauna of the Ellsworth Mountains⁸ has apparently been interpreted as having had unrestricted access to the open sea¹³.

The source of the fossiliferous erratics is unknown but all previous geologists who have worked in the area have considered that they have not moved far. However, it is difficult to support the suggestion that the source underlies the Blaiklock Glacier Group⁴, presumably in pockets. Wherever the base of the Blaiklock Glacier Group is exposed it rests unconformably on the basement rocks and leaves no room for intervening sedimentary beds. Also, no evidence of the fossiliferous material, as derived blocks, has been found in the rudaceous rocks of the Blaiklock Glacier Group and the latter are obviously derived directly from the underlying basement rocks. Therefore, the original suggestion by Stephenson¹ that the source of the fossiliferous erratics may be intermediate strata of the group hidden beneath Blaiklock Glacier seems more probable and supposes a marine transgression following deposition of the lower terrestrial beds, and preceding rejuvenation of the landscape with subsequent deltaic sedimentation¹⁵.

Radiometric Rb-Sr age data on basement rocks in this area range from 519 ± 15 Myr to 656 ± 66 Myr (E. S. Grew and M. Halpern, unpublished). Compared with the Middle Cambrian

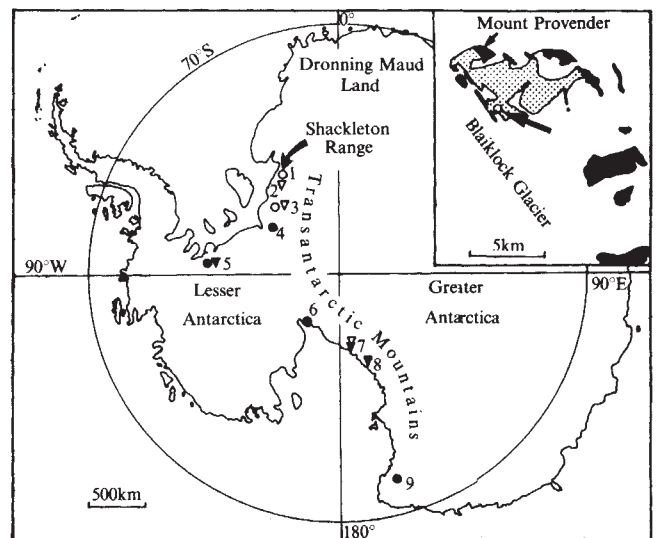


Fig. 1 Distribution of Cambrian faunas in Antarctica; circles denote shelly faunas, triangles denote archaeocyathid faunas (solid symbol = *in situ*, open symbol = moraine). 1, Mount Provender, Shackleton Range (Middle Cambrian); 2, Whichaway Nunataks (Lower Cambrian)²; 3, Argentina Range, Pensacola Mountains (Lower and Middle Cambrian)⁷; 4, Neptune Range, Pensacola Mountains (Middle Cambrian)⁷; 5, Heritage Range, Ellsworth Mountains (Upper Cambrian)⁸; 6, Harold Byrd Mountains (Middle Cambrian)⁶; 7, Beardmore Glacier area (Lower Cambrian)⁵; 8, Nimrod Glacier area (Lower Cambrian)⁵; 9, Evans Nève, northern Victoria Land (Lower and Upper Cambrian)^{9,10}. The inset shows the Mount Provender area of the Shackleton Range and the collecting locality of the specimens discussed here (bold arrow). Solid areas are rock outcrop, stippled areas are moraine.

palaeontological age for the fossils, this indicates fairly rapid uplift and erosion to expose the grade of metamorphism immediately underlying the conglomerates at the base of the Blaiklock Glacier Group. These conglomerates are almost certainly the products of that uplift and erosion.

If the fossiliferous material is very local in origin it can be concluded from the limited evidence available that the lower part of the Blaiklock Glacier Group pre-dates the fossiliferous erratics so that it is no younger than Middle Cambrian. An upper age limit for the upper part of the Blaiklock Glacier Group of pre-late Carboniferous is indicated by a dolerite dyke, intruding an outlier of the upper (deltaic) part of the group at The Dragons Back, 25 km to the east^{2,15}, which has a K-Ar age of 297 ± 12 Myr (ref. 16).

The occurrence of a slab with rounded edges (about 1.5 m in diameter by 0.4 m thick) in the moraine with one surface covered in broken brachiopod shells (P. D. Marsh, personal communication) suggests a third possibility, that the fossiliferous slabs are disintegrated remnants of large glacial erratic blocks from a distant source. However, no other exotic erratics have been found in the moraine so that it is still more likely that the debris has not moved very far.

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Opisthopubic pelvis in the carnivorous dinosaurs

THE dinosaurs are subdivided into Saurischia and Ornithischia on the basis of their pelvic girdles¹, which differ chiefly in the position of the pubis. In saurischians the pubis extends forward and downward whereas in ornithischians the supposed homologue of the pubis—the postpubis—is directed backward and downward parallel to the ischium. New finds from Mongolia, however, show that some carnivorous dinosaurs had a pubis orientated parallel to the ischium. As I report here, such a pubis has been found in the dromaeosaurids², the segnosaurids³ and a new genus from the Khara Khutul locality in

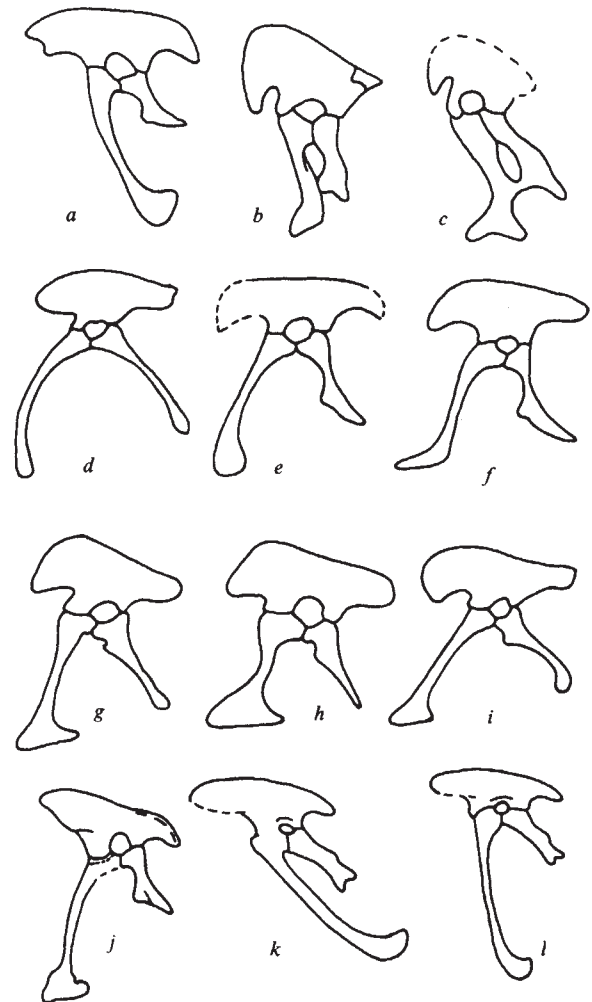


Fig. 1 Pelves of the carnivorous dinosaurs. *a*, Pelvis in the Mongolian dromaeosaurids; the same in: *b*, segnosaurids; *c*, dinosaur from Khara Khutul; *d*, podokesaurids; *e*, saurornithomimids; *f*, oviraptorids; *g*, allosaurids; *h*, Mongolian tyrannosaurids; *i*, ornithomimids; *j*, American dromaeosaurids (*Deinonychus*) as reconstructed by Ostrom (the pubis seems to be incompletely turned back); *k*, *Archaeopteryx* as traditionally reconstructed from Berlin specimen; *l*, *Archaeopteryx* as reconstructed by Ostrom (the pubis seems to be incompletely turned back as well in *Deinonychus*). The drawings in *b*, *d*, *g*, *j*, *k* and *l* are taken from refs 3, 9, 14 and 16.

south-east Mongolia, which has not yet been described and possibly represents a new family.

Dromaeosauridae⁴⁻⁶ are known from the Cretaceous of North America and Central Asia. In the Mongolian representatives of the family, *Velociraptor*^{5,7} and two genera not yet described, the pubis is directed backward⁸. In the North American *Deinonychus*⁶ the pelvis is not fully preserved and according to the most recent reconstruction⁹, the pubis seems to be incompletely turned back (Fig. 1*j*). The typical dromaeosaurid pelvis² (Fig. 1*a*) is characterised by a shallow ilium, a pubis with a broadly oval and laterally flattened distal end and an ischium significantly shorter than the pubis, with a large obturator process. Thus the dromaeosaurid pelvis has the general characteristics of the small theropods and only the direction of the pubis distinguishes it from the saurischian pattern (Fig. 1*d-l*).

The ilia in the segnosaurids (Fig. 1*b*) are well separated from each other and extremely deep, with very large anterior wings strongly deflected outward. The dorsal edge of the very short posterior iliac wing has a large cubic projection. It is not clear whether this projection should be considered homologous to the antitrochanter of ornithischians or whether it represents a new structure. The re-orientated pubis has a laterally flattened, large distal end; the ischium is elongated, its obturator process being

placed more distally than in dromaeosaurids and some other small theropods.

The preserved bases of the ilia of the Khara Khutul specimen (Fig. 1c) clearly show that these were also broadly separated and had cubic projections as in the segnosaurids. However, the pubis with the boot-like distal end is quite distinct from that in segnosaurids. The obturator process is situated distally, narrow and firmly coalesced with the pubic shaft, just above the distal end of the latter. Besides some common characteristics, the pelvis of the segnosaurids and the Khara Khutul specimen each have distinguishing features.

Thus, alongside dinosaurs with a typical saurischian pelvis, lived others with a pelvis not strictly 'saurischian'. I propose to refer to the former as 'prepubic' and the latter as 'opisthopubic', although these terms have already been used to designate two main pelvic structures in saurischians and ornithischians, respectively¹⁰. The opisthopubic pelvis probably provides evidence of evolutionary changes in the function and effectiveness of several pelvico-femoral muscles¹¹.

There is considerable similarity between the opisthopubic pelvis (particularly of the dromaeosaurids) and the primitive ornithischian¹² pelvis, reflecting to some extent the relationships of the two main types of pelvis and confirming the unity of the dinosaur stock¹³. On the other hand, the existence of the opisthopubic pelvis supports the idea that the parallel evolutionary lines of carnivorous dinosaurs² included some that transcended the limits of theropod evolution, within which predatory habits and full bipedalism¹⁴ were most effectively realised on the basis of a prepubic pelvis. The opisthopubic pelvis was evidently associated with specific lines of theropod evolution¹¹. Furthermore, the existence of this type of pelvis makes it more likely that *Archaeopteryx* had a posteriorly directed pubis^{15,16}.

In general, the opisthopubic pelvis must have had many advantages and probably appeared several times during both the early evolution of the dinosaurs and the ancestry of birds.

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Effect of salivary glands on wound contraction in mice

ALTHOUGH there has been speculation about the healing powers of saliva, recent interest in the salivary glands has been concerned with the physiology of nerve growth factor and epidermal growth factor^{1–4}. However, saliva also contains other biologically active substances such as kallikrein^{5,6}, amylase⁷, lysozyme⁸, immunoglobulins⁹ and renin¹⁰. Lysozyme is believed to enhance healing by suppressing infection¹¹. The wound healing process can be divided into epithelialisation, contraction, collagen synthesis and scar remodelling¹²: the evidence presented here suggests that contraction is affected by the salivary glands.

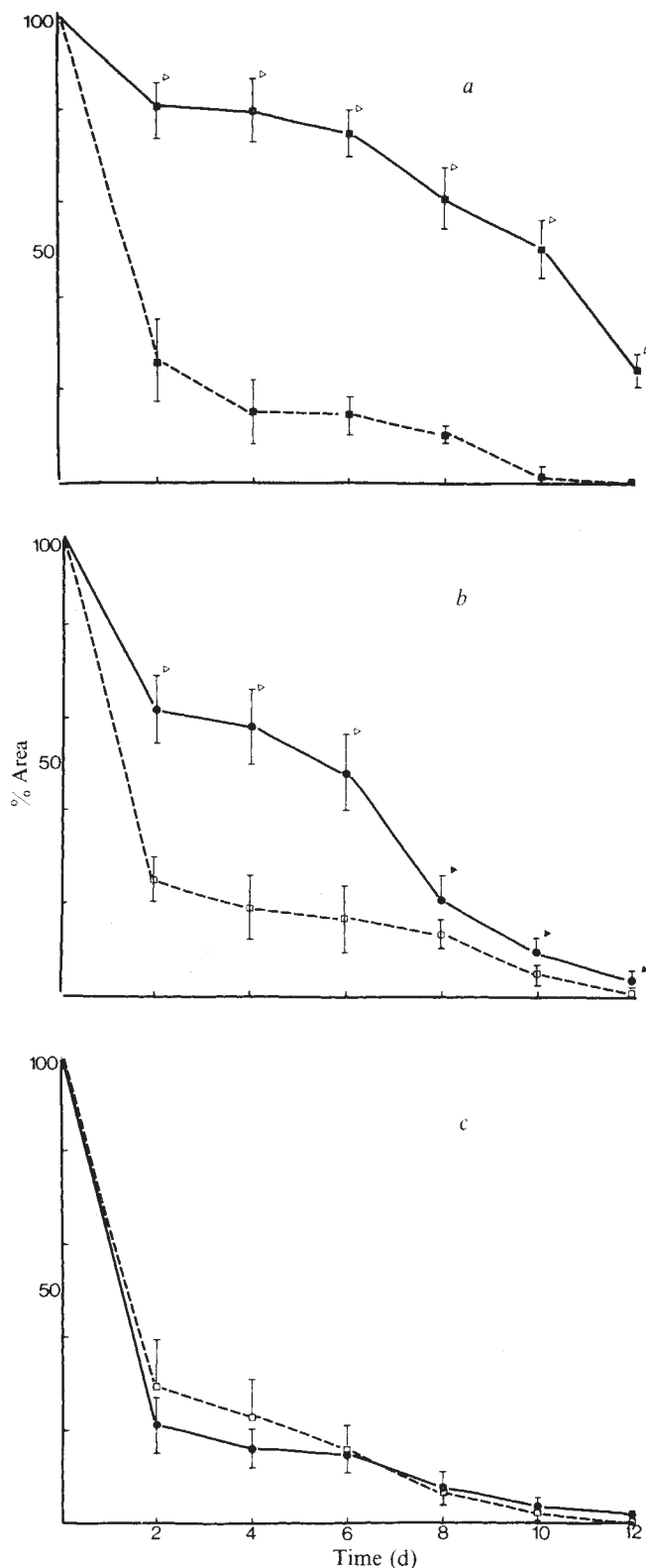


Fig. 1 Rate of subpannicular wound healing in normal mice caged separately or together (a), or after sialectomy (b, c). Results are expressed as mean % area $\pm$ 1 s.d. for five to six mice. a, Normal animals were caged either separately (—●—) or in groups (---□---). The healing rate was significantly faster with communal caging (Δ , $P < 0.001$). b, Mice caged in groups but with prior sialectomy (SMG, SLG) (—●—) or sham sialectomy (---□---). The rate of healing in sham-treated animals was the same as in non-operated controls (Table 1bII), but was significantly faster than in sialectomised animals (Δ , $P < 0.001$; $\blacktriangleright$, $P < 0.05$). c, Animals caged in groups with sialectomised (SMG, SLG) mice in the same box as sham-treated animals. In this situation no differences were found in healing rate ($P < 0.001$).

Table 1 The effect of various operative and non-operative procedures on the rate of wound healing

Experimental groups		No. of animals	No. of days after wounding					
			2	4	6	8	10	12
<i>a, Suprapannicular wounds</i>								
I	Controls (separated)	4	92±2	81±10	65±17	55±19	24±7	8±6
II	Controls (together)	10	37±15	25±8	19±7	10±2	4±2	2±1
III	Sham sialectomy	7	39±7	26±5	19±5	11±4	4±1	1±1
IV	Sialectomy (SMG, SLG)	15	96±15	78±14	58±13	30±9	12±5	5±1
V	Sialectomy (SMG)	5	55±6	48±8	32±9	19±3	14±4	2±2
VI	Sialectomy (parotid)	5	47±6	39±2	21±4	13±3	6±3	1±1
VII	Duct ligation (no delay)	6	79±15	59±11	49±6	18±6	9±9	3±3
VIII	Duct ligation (4-day delay)	6	64±7	56±9	42±5	22±5	7±2	3±2
IX	Duct ligation (7-day delay)	7	70±4	64±5	53±3	27±4	—	5±1
<i>b, Subpannicular wounds</i>								
I	Controls (separated)	6	81±5	80±7	75±5	61±7	50±6	23±4
II	Controls (together)	5	26±9	15±7	14±4	10±2	1±2	0
III	Sham sialectomy	5	25±5	19±7	17±7	14±3	5±2	1±2
IV	Sialectomy (SMG, SLG)	5	62±7	58±8	48±8	21±5	10±3	3±2
V	Shams (with sialectomies)	6	29±10	23±8	16±5	7±3	2±2	0
VI	Sialectomies (with shams)	6	21±6	16±4	15±4	8±3	3±2	1±1

The wound areas were traced on to paper using a transparent plastic sheet; the paper was cut and then weighed on a Mettler H54 balance. The weight of the tracing taken 2 h after wounding was arbitrarily designated as 100, and all subsequent measurements were expressed as a percentage. All sheets of paper came from the same batch and were of uniform weight (12.5 ± 0.03 g) for their size of $43.2 \text{ cm} \times 27.5 \text{ cm}$. Measurements were made on alternate days up to 12 d when further accurate measurements were not possible. The results were compared using a *t*-test for the mean % area (± 1 s.d.). Animals were housed in groups of four to six during the experiments except in groups aI and bI. Sialectomy, carried out 7 d before the skin wound, included the submaxillary glands (SMG) and sublingual (SLG) or parotid glands as indicated. Ligation of the submaxillary and sublingual gland ducts was carried out with intervals of 0, 4 or 7 d before wounding.

A 1-cm^2 skin wound was cut into the centre of the back in 110 female C57BL/6J mice aged 50–100 d. Both superficial and deep wounds were studied; the superficial wounds were made by excision of skin only and the deep wounds by excision of skin and panniculus carnosus muscle together. The wound was allowed to dry and retract for 2 h before initial measurement. At 48-h intervals after wounding, the animal was lightly anaesthetised with ether, and the wound area measured. Table 1 summarises the measurements made at 0–12 d in each group. Histological sections were prepared from six controls and seven sialectomised animals on alternate days up to 12 d. Paraffin sections were prepared with both haematoxylin and eosin or Gomori Trichrome.

In normal mice, healing rate of suprapannicular (Table 1aI, II) and subpannicular (Table 1bI, II) wounds were almost the same, the deeper subpannicular wounds showing a slightly faster rate of contraction during the first 48 h. Wound healing in normal mice varied with the caging method (Fig. 1a). When the animals were caged separately their wounds healed slowly, with

the formation of a thick eschar after several days. The mice attempted to lick their own wounds but this was prevented by the wound position in the centre of the back. When the animals were caged in groups of four to six, all the wounds underwent marked contraction within 1–4 d. The initial square wounds became stellate, similar to patterns described in rabbits¹³ and guinea pigs¹⁴ (Fig. 2). Communal grooming and wound licking were observed whenever animals were housed together, and therefore all subsequent experiments were carried out with grouped mice.

After removal of both the submaxillary and sublingual glands (SMG, SLG), the contraction rate was significantly slower than in sham-operated animals ($P < 0.001$), despite wound licking (Fig. 1b). The delayed contraction was similar to that observed in normal animals caged alone, with little change during the first 4 d. The sham-sialectomised mice showed rapid wound contraction similar to non-operated controls ($P > 0.05$).

When sialectomised (SMG, SLG) and sham-operated animals were caged together (Fig. 1c), permitting communal wound licking, the healing rates were not significantly different ($P > 0.05$), with all animals showing obvious early wound contraction.

After removal of the submaxillary glands alone, contraction of wounds was slower than in sham-operated controls ($P < 0.01$, except day 12). However, contraction was even slower when both submaxillary and sublingual glands were removed ($P < 0.05$, except day 10). This result implied that the relatively small sublingual glands, as well as the large submaxillary glands, contained an active substance affecting wound contraction.

Following bilateral parotidectomy the healing rate of wounds approached that observed in sham-operated controls ($P > 0.05$, except day 4), with contraction macroscopically indistinguishable from that of controls. This suggested that the parotid glands had no significant role in the effect.

To examine the possibility that the healing effect may be mediated by both exocrine and endocrine routes, experiments with animals which had undergone gland-duct ligation were carried out (Fig. 3). Despite intervals of 0, 4 or 7 d between duct ligation and wounding, healing patterns were not significantly different ($P > 0.2$), and therefore a composite group is shown. Healing was significantly slower than in either non-operated or sham-sialectomised controls ($P < 0.001$), until day 8. When

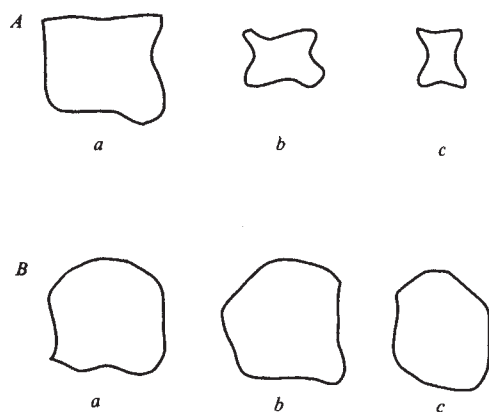


Fig. 2 Effect of removal of submaxillary and sublingual glands on early wound shape. Aa–c Shows the rapid wound contraction seen in a normal animal at 0, 2 and 4 d, respectively. A wound of a sialectomised animal at 0, 2 and 4 d is shown in Ba–c. The wound outlines were traced from photographs, and are approximately $\times 2$ actual size.

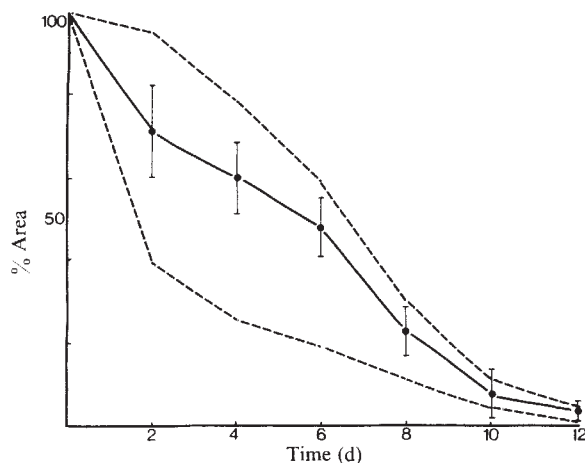


Fig. 3 Effect of ligation of submaxillary and sublingual ducts on rate of suprapannicular wound healing. Results are expressed as mean % area $\pm$ 1 s.d. for 19 mice (only 12 mice on day 10). *t*-Test showed no statistical difference between the duct-ligated groups despite intervals of 0, 4 or 7 d before wounding, and therefore this figure shows pooled data (●—●). The sialectomised (SMG, SLG) (Table 1aIV) and sham-operated animals (Table 1aIII) are shown as 'ghosts' (----) for comparison. Wound area was significantly smaller than after sialectomy (SMG, SLG) until day 8 ($P < 0.05$), although it was also larger than in controls until day 8 ($P < 0.0001$).

compared with sialectomised (SMG, SLG) animals, the wounds of the duct-ligated group were significantly smaller up to day 9 ($P < 0.05$), suggesting that saliva was required for rapid wound contraction, and also the possibility of an early endocrine-mediated response. Even after sialectomy, epithelialisation was apparently normal on histological study, but the cause of delayed contraction was not determined.

The rate of wound contraction observed in this study is greater than that reported by other workers¹⁵, although animals have rarely been permitted or observed to lick their wounds¹⁶. We considered that the sialectomy wound might itself affect subsequent wound healing, although evidence of different healing rates between primary and secondary wounds is controversial¹³. However, as wounds in non-operated and sham-operated controls contracted equally fast, this possibility can be discounted.

The difference between wounded mice caged individually or in groups was the occurrence of communal wound licking, suggesting an effect either of licking itself or of a salivary gland factor. The mechanical action of the tongue, by minimising scab thickness, could reduce any splinting effect of the scab delaying wound contraction. The scab was certainly thinnest in communally licked controls (or sham-operated animals) with the fastest contraction, although this occurred in the first 2–4 d, before the scab became protuberant. The marginally faster healing in sialectomised animals caged together, compared with controls caged individually, suggested that there was a slight mechanical effect; however, any major effect was unlikely in view of the much slower healing observed in sialectomised animals licked by sialectomised companions than in controls similarly grouped.

Recent evidence suggests that contraction may be controlled by myofibroblasts within granulation tissue¹⁷. These modified fibroblasts contain smooth muscle-like contractile proteins as well as endoplasmic reticulum for collagen synthesis¹⁸. Contraction can be experimentally inhibited by smooth-muscle antagonists¹⁹, but there are no reported methods of its acceleration.

It is uncertain whether sialectomy (SMG, SLG) completely or only partially inhibited contraction; also, the mechanism by which contraction was affected is unknown. Vasoactive substances may accelerate the inflammatory response; kallikrein, which stimulates smooth muscle²⁰, may also be involved

in myofibroblast contraction. Alternatively, an unrecognised salivary gland factor may facilitate fibroblast migration, or the differentiation of myofibroblasts, although the origin of these cells is still uncertain²¹.

These results suggest that the mouse submaxillary and sublingual salivary glands contain a factor(s), largely applied by licking, which accelerates early wound contraction. This may be of practical significance in human surgery, as wound contraction is believed to be similar in all mammals. The panniculus carnosus in animals merely allows faster contraction because of greater skin mobility¹². The next step towards a practical use of our finding would be the isolation of the salivary gland healing factor postulated here.

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Normalisation of sister chromatid exchange frequencies in Bloom's syndrome by euploid cell hybridisation

BLOOM'S syndrome (BS) is a rare autosomal recessive genetic disorder characterised by stunted growth, Sun-sensitive telangiectatic erythema of the face and an abnormally high risk of cancer¹. Cultured lymphocytes and fibroblasts from BS patients have a high frequency of spontaneous structural chromosome aberrations and characteristically high levels of sister chromatid exchanges (SCE)². The primary defect at the molecular level is unknown, but the cytological findings are compatible with a deficiency in a DNA repair function³. The presence of a defect of DNA replication is also suggested by the demonstration that the rate of fork motion during replication in BS is slower than normal⁴. German *et al.*⁵ have reported the coexistence of cells with both high and normal levels of SCE, suggesting that the defect in BS is regulatory. Tice *et al.*⁶ concluded from co-cultivation experiments that SCE frequency is mediated by a diffusible agent(s) present in the medium of BS fibroblasts. Other investigators, however have shown suppression of SCE in conditions of co-cultivation^{7,8}. We have clarified the conflict

between these results by examining euploid somatic cell hybrids between BS and normal human fibroblasts.

In the absence of selectable biochemical markers, we obtained hybrids by visual identification of tetraploid clones emerging from fusogen-treated cell mixtures^{9,10}. Equal numbers of both parental cell types were co-cultivated for 36 h and then treated with a combination of polyethylene glycol and dimethyl sulphoxide (DMSO)¹¹. After 10–12 days presumptive tetraploid colonies were located by phase contrast microscopy¹², isolated and screened electrophoretically for heteropolymeric glucose-6-phosphate dehydrogenase (G6PD)¹³. Cytogenetically, hybrid clones were tetraploid with two Y chromosomes of different size and heterochromatin content. A reversion to normal levels of SCE in the BS parental phenotype (Fig. 1a and b) was noted in six independent hybrids isolated. Only three of these hybrid clones could be propagated sufficiently for subsequent quantitative analysis of the frequency of SCE. Additional metaphases were classified subjectively as 'low SCE' or 'high SCE', corresponding to the normal and the BS parental SCE phenotype, respectively. Results of these quantitative and qualitative analyses (Table 1) demonstrate that fusions of mutant (high frequency of SCE) with normal (low frequency of SCE) genomes result in complete correction of the mutant phenotype. In contrast, tetraploid clones resulting from fusion between BS parental cells retained the characteristic high frequency of SCE (Table 1).

The possibility that the observed normal low levels of SCE in the hybrids could arise from preferential fusion of the low SCE parent with a subset of BS cells which have low levels of SCE is unlikely for two reasons. First, there was no evidence for bimodality⁴ within the BS fibroblast metaphases as regards levels of SCE: the parental BS cells uniformly showed characteristic high levels of SCE (Table 1). Second, in repeated collections, 20–25% of the BS parental metaphases included a small centric fragment derived from an apparently stable rearrangement involving acrocentric chromosomes. These cells were able to form complementing hybrids with normal cells, as observed in hybrid 24, which contained the centric fragment in all metaphases analysed. Thus, at least two karyotypically distinctive subsets of cells from this BS strain had SCE phenotypes which were recessive in hybrids with normal cells. In addition,

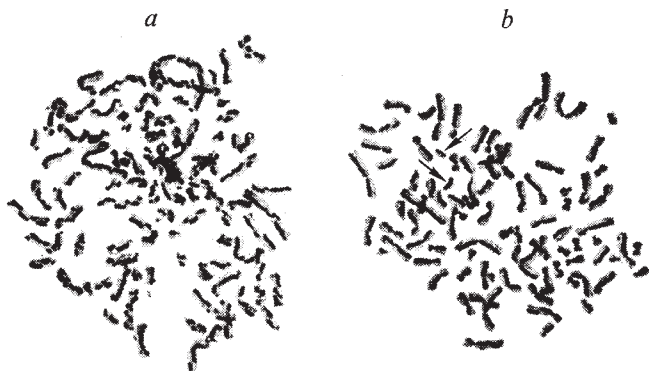


Fig. 1 Cell cultures were grown in Waymouth media supplemented with 16% heat-inactivated FCS. The same lot of serum was used throughout the experiment to control for variation in levels of spontaneous SCE due to serum¹⁵. The BS strain (GM 1492; Human Genetic Mutant Cell Repository, Camden) was G6PD type B, had a variant size Y chromosome (q12; CBG40)¹⁶ and had chromatid gaps, breaks and rearrangements in more than 16% of its metaphases in three subsequent collections. The G6PD type A strain was derived in our laboratory from neonatal foreskin. For analysis of SCE, cells were exposed to BUdR (3 µg ml⁻¹) for 44 h in 75-cm² tissue culture flasks. Colcemid (0.04 µg ml⁻¹) was added 2 h before collection and slides were prepared according to standard cytogenetic techniques. Differential staining of chromatids was accomplished with heat and Giemsa according to the method of Korenberg and Freedlander¹⁷. *a*, G6PD B type (BS) tetraploid metaphase, showing high SCE phenotype. *b*, hybrid metaphase with low SCE phenotype. Arrows point to the discordant Y chromosomes.

Table 1 Quantitative and qualitative evaluation of SCE frequencies in parental, diploid, tetraploid and hybrid cultures

Cultures/Clone	G6PD type	No. of SCE per metaphase*	SCE phenotype	
			Low	High
BS parental diploid (GM 1492)	B	75.4 ± 8.9 (22)	(0)	(150)†
Normal parental diploid (74–30)	A	7.0 ± 1.8 (25)	(50)	(0)
Hybrids				
No. 6	AB	12.4 ± 2.7 (15)	(17)	(0)
No. 9	AB	10.4 ± 2.3 (17)	(22)	(0)
No. 24	AB	9.2 ± 1.8 (15)	(20)	(0)
BS tetraploids‡				
No. 15	B	129.2 ± 9.5 (17)	(0)	(25)
No. 28	B	133.1 ± 11.4 (16)	(0)	(20)
Normal tetraploids				
No. 58	A	10.5 ± 1.5 (6)	(19)	(0)

AB represents the G6PD heteropolymeric enzyme.

*Values are means ± s.d.; numbers of metaphases are shown in parentheses.

†50 Metaphases from each of three independent collections.

‡Isolated concomitantly with hybrid tetraploid clones after fusion treatment and dilution plating.

none of the hybrid clones seemed to display the characteristically high levels of unstable chromatin aberrations of BS cells in first division metaphases (defined by the staining response following incorporation of bromodeoxyuridine (BUdR)). The latter observations require quantification in a larger series of hybrids.

Our results do not support the evidence of Tice *et al.*⁶, which suggested that an endogenous production of a DNA-damaging agent caused the high frequency of SCE in BS cells. Rather, they are consistent with the idea that the high frequency of SCE reflects an intrinsic genetic defect in BS cells themselves.

Metaphases from BS heterozygotes show normal levels of SCE². We plan to determine the level of SCE in hybrids between BS heterozygotes and related BS homozygotes. Such hybrids would contain three abnormal but only a single normal allele for the as yet unidentified gene defect. If such hybrids display intermediate levels of SCE, they would serve as a useful test system for heterozygosity¹⁴. Our technique of euploid–euploid hybridisation might, in addition, be used to investigate the question of genetic heterogeneity among BS patients¹.

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Human brain tumour cell strains with deficient host-cell reactivation of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-damaged adenovirus 5

HOST-CELL reactivation of a population of physically or chemically damaged viruses is said to occur when the plaque titre of the damaged (but not the control) virus population depends strongly on the cell strain selected to serve as the host to support virus growth^{1,2}. Such cell strain-dependent differences in survival have classically been interpreted as reflecting known cellular differences in ability to repair DNA^{1,2}. For example, we have previously demonstrated host-cell reactivation of UV-irradiated³ or benzo(a)pyrene diol-epoxide I (anti)-treated⁴ human adenoviruses. The survival of such treated virus populations is much greater when their plaque titre is measured using monolayers of fibroblasts with normal DNA repair than when using fibroblasts from patients having the genetic disease, xeroderma pigmentosum, which are deficient in repair of UV-damaged DNA. The work of Lytle and coworkers^{5,6} and Day³, using human cells, has shown host-cell reactivation of UV-damaged viruses to occur only when nuclear replicating DNA viruses are studied. To assess the hypothesis that human tumorigenesis is often associated with repair-deficient cells, we undertook a study of host-cell reactivation of various kinds of damage using human cell strains prepared both from tumours of different organs and from skin of people having genetic predisposition to, or familial occurrence of, cancer. As part of this work, we measured the survival of adenovirus 5 treated *in vitro* with the carcinogen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). We found that the use of cells from 4 of 13 human brain tumour strains as viral hosts resulted in less survival of the MNNG-damaged viruses than did the use of more than 20 other cell strains prepared from human tumours or from unaffected human organs (Fig. 1, Table 1). Previous studies have not detected abnormal DNA repair in human tumours⁷. We believe this to be the first report of host-cell reactivation of MNNG damage by mammalian cells.

Data were obtained in eight experiments with A172 (Fig. 1, data from six) and in two each with A382, U-87MG and U-105MG. A172 was established from a glioblastoma⁸ (a glioblastoma is a highly malignant (Grade IV) astrocytoma), the other three from astrocytomas⁸⁻¹⁰. Six other cell strains (T-98, 118MG, U-373MG, U-251MG, U-178MG and U-138MG) also established from glioblastomas or astrocytomas^{9,10} showed approximately the same level of host-cell reactivation as the 23 remaining strains listed in Table 1 (data obtained using three of the six are included in Fig. 1). Among the strains showing normal reactivation of MNNG-damaged adenovirus 5 were three skin fibroblast strains prepared from patients having ataxia telangiectasia—AT3BI, AT5BI and AT81CTO (inactivation data for the first two of these is given in ref. 11). The colony forming ability of strain AT3BI has been found to be more sensitive to inactivation by MNNG than that of the KD, CRL1187, and CRL1220 normal strains¹². One cell strain from a xeroderma pigmentosum patient, XP12BE, previously shown to be highly defective in host-cell reactivation of both UV-damaged³ and benzo(a)pyrene diol-epoxide I (anti)-damaged⁴ adenovirus 5, shows a normal level of host-cell reactivation of MNNG-damaged virus. Three skin fibroblast cell strains from persons with brain tumours, and belonging to cancer-prone families¹³⁻¹⁵, also show normal reactivation of the MNNG-damaged virus.

Although A172, U-87MG and U-105MG host cells reactivate MNNG-damaged adenovirus 5 poorly, Fig. 2 shows that they have normal host-cell reactivation of UV-irradiated viruses. The UV sensitivity of the virus reflected by the slope of the inactivation curve is experimentally indistinguishable from

that previously reported using 10 normal human skin fibroblast strains³. Thus the defective host-cell reactivation of MNNG-damaged adenovirus 5, characteristic of these strains, does not reflect a general decrease in their ability to support the growth of damaged adenoviruses. (While A172, U-87MG and U-105MG grow very well, the slow growth of A382 has slowed further experimentation, and UV survival was not tested with this strain.)

We interpret our results to mean that the A172, A382, U-87MG and U-105MG cell strains are defective in the ability to repair MNNG-damaged adenovirus 5. While we believe the MNNG lesion, which is not repaired well by these strains, to be

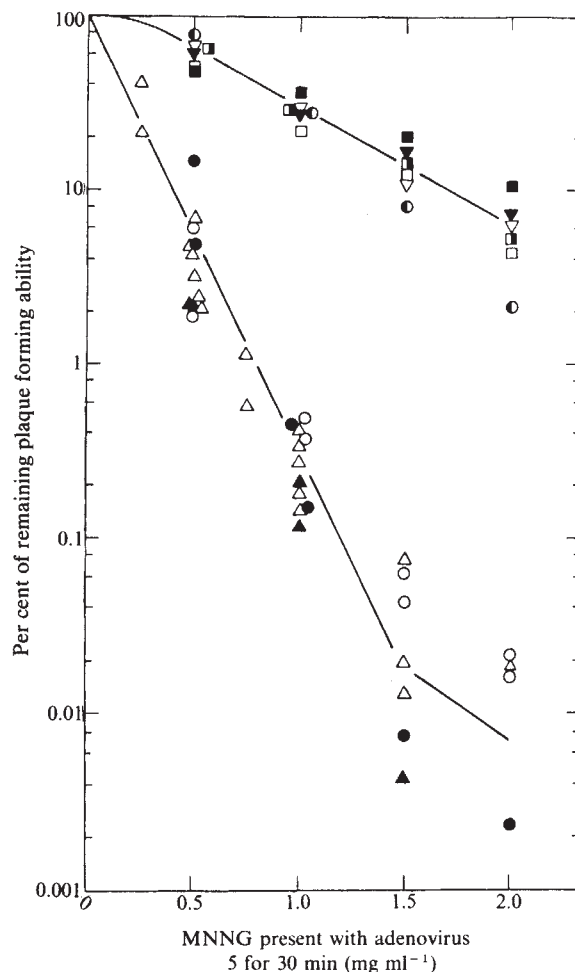


Fig. 1 Per cent remaining plaque forming ability of adenovirus 5 as a function of MNNG concentration present with the virus. Human adenovirus 5, purified as previously described⁴, was diluted 1:1000 in 0.3 M Tris, pH 9.0. Aliquots (0.1 ml) of ethanol stock solutions of appropriate concentrations of MNNG (Aldrich) were added to 0.9-ml portions of the diluted virus suspension. After 30 min incubation at 37 °C, 0.1 ml of 0.5 M *N*-acetyl-L-cysteine¹⁶, pH 7.2, was added to each treated virus sample to reduce the remaining MNNG and thereby to terminate the viral inactivation. After 5–15 min further incubation, samples were diluted 1:10 into basal medium Eagle's medium (BMEM) (Microbiological Associates) containing 1% fetal bovine serum (GIBCO) plus 50 U penicillin and 50 µg streptomycin per ml. Plaque assays were then carried out as described previously^{3,4,20}. The strains used, followed by the respective number of adenovirus 5 plaque forming units per ml from the dilution of non-treated viruses into BMEM were: ▲, A382, 11.4×10^5 ; ●, U-87MG, 30.2×10^4 ; ■, U-373MG, 50.8×10^4 ; ○, U-105MG, 45.4×10^4 ; □, U-178MG, 53×10^2 ; △, A172, 36.3×10^4 ; ▼, H4, 90.8×10^4 ; ▽, 118MG, 92.4×10^3 ; ●, CRL 1187, 16.2×10^4 ; and ■, HEK, 13.5×10^5 . Several points are displaced for clarity. Further cell strain data are given in Table 1 legend.

Table 1 Cell strains used

Human tumour cell strains	Tumour of origin	Donor age, sex	Skin fibroblast cell strains	Characterisation	Donor age, sex
Reactivation deficient			Reactivation proficient		
A172	Glioblastoma (astrocytoma, grade IV)	53, M	CRL 1220	Apparently normal	15, M
A382	Astrocytoma	10, M	CRL 1187	Apparently normal	14, M
U-87MG	Astrocytoma, grade II	54, F	CRL 1224	Apparently normal	40, F
U-105MG	Astrocytoma, grade III	62, M	KD (CRL 1295)	From blood blister of lip	31, F
			WR001	Cockayne's syndrome	9, F
Reactivation proficient			XP12BE (CRL1223)	Xeroderma pigmentosum	7, F
118MG	Glioblastoma vs. astrocytoma	50, M	AT3BI	Ataxia telangiectasia	4, M
U-373-MG	Astrocytoma, grade III	61, M	AT5BI	Ataxia telangiectasia	?, M
U-251MG	Astrocytoma, grade I	75, M	AT81CTO	Ataxia telangiectasia	<1, M
U-178MG	Astrocytoma, grade II	56, M	AL1405	Acute myelogenous leukaemia	6, M
U-138MG	Glioblastoma	47, M	AL639	Acute monomyelogenous leukaemia	—
T-98	Glioblastoma	61, M	AL377	Brain tumour prone family	19, M
Hs783T	Neuroblastoma	4, M	AL2673	Brain tumour prone family	38, M
Hs683	Glioma	76, M	AL1899	Brain tumour prone family	35, M
H4	Neuroglioma	37, M			
A498	Kidney carcinoma	52, F	Other cell strains		
A704	Kidney carcinoma	78, M	Reactivation proficient		
Hs775T	Wilms tumour (kidney)	13, M	HEK	Human embryonic kidney	—
Hs703	Liver carcinoma	55, M			
A204	Rhabdomyosarcoma	1, F			
			Per cent of remaining plaque forming ability		

Cell strains A172, A204, A382, A498 and A704⁸, 118MG, H4, Hs783T, Hs683, Hs703 and Hs775T were from Dr Walter Nelson-Rees and Jack Weaver, with support from the NCI/Viral Oncology program, under the auspices of the Office of Naval Research and the Regents of the University of California. Cell strains T-98, U-87MG, U-105MG, U-138MG, U-178MG, U-251MG and U-373MG were from Dr Jorgen Fogh, Sloan-Kettering. Strains designated by 'CRL' were from the American Type Culture Collection, Rockville. HEK cells were from GIBCO. Strains designated AL were supplied by Dr Anthony Lubiniecki, Meloy (AL377 is from case IV-7 in NIH Clinical Epidemiology Branch family 0068 (ref. 13), AL1899 is from case 3 in family 0272 (ref. 14), AL2673 is from case V-8 in family 0165 (ref. 15), and AL1405 from family 0827). AT3BI and AT5BI were from Dr Colin Arlett and AT81C10 was from Dr M. C. Paterson, Atomic Energy of Canada. Strain WR001 was the gift of Dr Peter Nissley, NCI, NIH. Strain XP12BE belongs to xeroderma pigmentosum complementation group A (ref. 21).

in the DNA of a damaged adenovirion, the identification of this lesion as being an MNNG altered viral protein remains a possibility, although there are no reports of successful cellular repair of damaged proteins.

Candidates for repairable lesions produced by MNNG in adenovirus DNA are methylated bases¹⁶, phosphotriesters¹⁷ and *N*-nitrocyanamide reaction products¹⁸. In our experiments, we activated MNNG by low amounts (pH 9) of hydroxyl ion, a reaction which gives methyl diazonium hydroxide (which subsequently becomes a methylating agent) and *N*-nitrocyanamide anion¹⁶. We have found that another type of virus treatment (treatment of virus suspensions by reduction of MNNG using *N*-acetyl-L-cysteine¹⁶) which produces a strong methylating agent, but does not produce active *N*-nitrocyanamide anion¹⁶, does not result in viral inactivation. This, by elimination, suggests that the repairable lesion may be a DNA-*N*-nitrocyanamide reaction product. Further results suggesting the possible importance of the *N*-nitrocyanamide moiety come from comparative studies of the kinetics of viral inactivation using MNNG and its ethyl counterpart, ENNG. Although these two agents produce different amounts of alkylated bases and phosphotriesters relative to their total reaction¹⁷ (MNNG gives more total reaction than ENNG), they give the same viral inactivation kinetics per mol using repair proficient cells (data not shown), suggesting that the viral inactivating lesion is due either to the *N*-nitrocyanamide group, common to the two agents, or to some alkylated base or phosphotriester made in quantities such that the biological effects of the two agents are equal.

The fact that the decreased survival of MNNG-treated virus is observed in four of ten cell strains prepared from human astrocytomas suggests four possibilities for the relationship of MNNG host-cell reactivation deficiency to the origin of the astrocytomas that gave rise to the A172, A382, U-87MG and U-105MG cell strains: (1) tumorigenesis leading to astrocytomas is often initiated in a group of cells which is normally

deficient in host-cell reactivation of MNNG-damaged adenovirus; (2) such tumorigenesis often generates host-cell reactivation deficient cells; (3) such tumorigenesis is initiated in a cell, which, by somatic mutation, has become host-cell reactivation deficient; and (4) a large proportion of astrocytomas

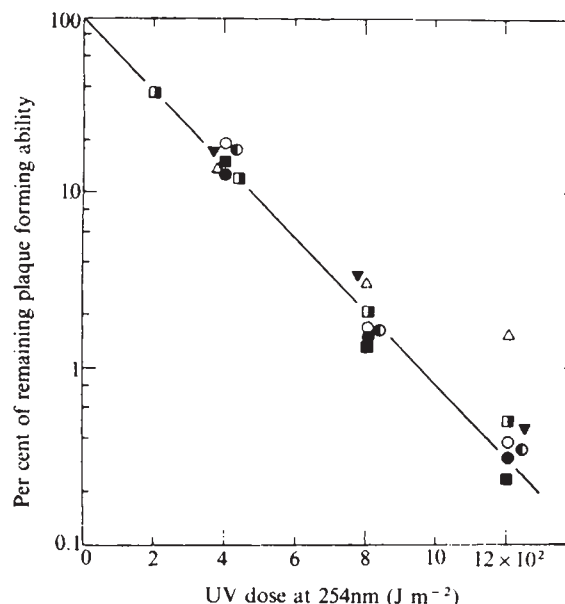


Fig. 2 Per cent remaining plaque forming ability of adenovirus 5 as a function of UV dose to the virus. Purified human adenovirus 5 was diluted 1:1,000 in PBS, UV-irradiated, and assayed for plaque forming ability^{3,4,20}. The strains used, followed by the number of adenovirus 5 plaque forming units per ml PBS were: ●, U-87MG, 18.0×10^5 ; ○, U-105MG, 30.4×10^5 ; △, A172, 84.7×10^4 ; ▼, H4, 22.6×10^6 ; ○●, CRL 1187, 29.9×10^4 ; and ■, HEK, 21.6×10^6 . See Table 1 legend for further strain data.

arise in people who have a genetic defect manifested by impaired host-cell reactivation of MNNG-treated adenoviruses. If the last possibility were correct, the disease identified would be another of the genetic diseases in which defective cellular repair of damaged DNA is associated with carcinogenesis. The three diseases known to be of this type, xeroderma pigmentosum, ataxia telangiectasia and Fanconi's anaemia, are infrequently associated with brain tumours¹⁹.

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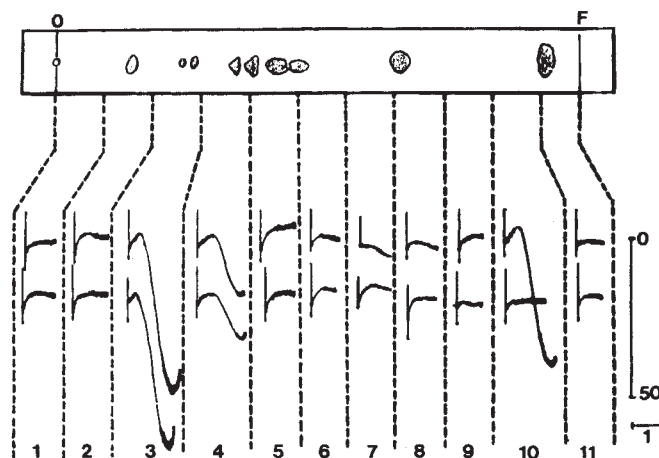


Fig. 1 Separation by TLC of aggregating substances released during ionophore-induced platelet aggregation. The upper part of the figure shows the thin layer chromatogram obtained by running a 10-μl aliquot of the 100 μl of the chloroformic platelet lipid extract. Spots were revealed with iodine vapour. The 90 μl remaining was run in parallel and divided into 11 square zones. Aliquots of 1 μl of the eluate from each zone were added to 0.35 ml of rabbit washed platelets (5×10^8 platelets per ml) incubated for 15 s with (lower tracings) or without (upper tracings) 5 μM indomethacin. Two distinct areas were detected: eluates from zones 3 and 4 (left hand) induced aggregation which was unaffected by indomethacin and can thus be considered as cyclo-oxygenase independent. Eluate from zone 10 (right hand) induced aggregation which was completely blocked by indomethacin, indicating the presence of arachidonic acid. Vertical scale: % light transmission; horizontal scale: time in minutes.

Arnoux and D. Duval, in preparation) macrophages. Moreover, thrombin and ionophore-induced platelet aggregation and the accompanying stimulation of phospholipase A_2 (refs 13, 14) are inhibited by the phospholipase inhibitor bromophenacyl bromide (ref. 15 and B.B.V., F. Fouque and M.C., in preparation), suggesting that the third mechanism of platelet aggregation might involve a lipid mediator. These findings prompted us to investigate whether platelets can form and release PAF in experimental conditions in which the ADP and TXA_2 pathways are fully blocked. We report here that this is indeed the case, and suggest PAF as a likely candidate for mediating the 'third pathway' of platelet aggregation.

In 12 experiments, blood was collected in EDTA (2.5 mM) from the ear artery of rabbits, and platelet-rich plasma obtained by standard centrifugation procedures. Washed platelets were prepared according to the method of Ardlie *et al.*¹⁶ modified as described elsewhere⁶. In two experiments human blood was collected on citric acid–citrate–dextrose (for 9 ml of blood, 1 ml of a solution made of 8 g citric acid monohydrate, 22 g citrate trisodium dihydrate and 22.3 g glucose per l of distilled water); platelets were washed by two centrifugations at 1,000g for 15 min on a cushion of erythrocytes and then separated by centrifugation at 90g for 15 min (modified by J.B. from ref. 17). Washed platelets from rabbits (5×10^9) and from humans (3×10^9) were suspended in 10 ml of Tyrode's solution containing 2.5 g l⁻¹ bovine serum albumin (fraction V, Armour) and buffered with Tris (Sigma), 0.01 M, at pH 7.4. Platelets were stirred at 37 °C with or without 2.5 μM A23187 (Lilly). In four experiments aspirin (Prolabo) and the CP/CPK enzymatic system were added to platelets 10 min before the ionophore. In every experiment, the concentrations used, 0.1 mM for aspirin and 31.25 μg ml⁻¹ and 15.25 μg ml⁻¹ for CP and CPK (Sigma), respectively, were able to block aggregation induced by 0.1 mM arachidonic acid and to convert 50 μM ADP after 1 min incubation.

Ten minutes after the addition of the ionophore the suspension was ultrasonicated for 30 s in a Branson ultrasonicator, and the pH was adjusted to 10.6 with NaOH. Incubates were stirred

The role of platelet-activating factor in platelet aggregation

PLATELET aggregation is mediated by at least three distinct mechanisms^{1,2}. The first involves the release of ADP and is inhibited by its conversion to ATP by the combination of creatine phosphate and creatine phosphokinase (CP/CPK). The second is mediated by metabolites of arachidonic acid, particularly thromboxane A_2 (TXA_2), and is blocked by aspirin or indomethacin, inhibitors of the arachidonate cyclo-oxygenase pathway³. It has been postulated that a third mechanism must exist, as neither CP/CPK nor aspirin, alone or combined, can inhibit aggregation induced by high concentrations of thrombin or the calcium ionophore A23187 (refs 1, 2, 4). Antigenic challenge of IgE-sensitized basophils releases a platelet-activating factor (PAF), probably a 1-lysophosphatidylcholine⁵. PAF has a potent action on rabbit^{6,7} and human^{8,9} platelet aggregation and release which is independent of the cyclo-oxygenase arachidonate pathways^{6,10,11}. We have also obtained PAF from A23187-stimulated rat peritoneal¹² and alveolar (J.B., B.

Table 1 Release of platelet-activating factor and arachidonic acid from rabbit and human platelets during A23187-induced aggregation

		Additions		A23187 Aspirin and CP/CPK
		None	A23187	
Rabbit	PAF	0	1,957 ± 1,147	1,797 ± 740
	AA	756 ± 576	1,197 ± 653	732 ± 282
Human	PAF	0; 0	625; 725	ND
	AA	0; 0	145; 130	ND

Washed platelets were incubated for 10 min with or without A23187, a combination of aspirin and CP/CPK having been added 10 min before the ionophore (see text). Numbers represent platelet-aggregating activities expressed in arbitrary units per ml of incubate, recovered after TLC. PAF, activities from zone 3, indomethacin resistant; arachidonic acid (AA), activities from zone 10, indomethacin sensitive. Results of rabbit experiments are the mean ± s.d. of 12 experiments on 5×10^8 platelets per ml; those of humans are from two separate experiments using 3×10^8 platelets per ml. Note that arachidonic acid was also released from platelets used as controls, probably due to damage during extraction. ND, not done.

for 1 h at room temperature and 1.5 volumes of absolute ethanol were then added. The mixture was evaporated overnight under a stream of air and the residue was extracted twice with 1 ml of chloroform. After evaporation the new residue was dissolved in 100 μ l of chloroform. Samples were spotted on silica-gel TLC plates (Merck, 60F-254) which were developed with a chloroform/methanol/water mixture (70:35:7). The plates were divided into 11 squares of 1.5×1.5 cm, and the gel from each square was scraped and eluted with 500 μ l of 60% ethanol. Eluates, usually 1- μ l aliquots, were assessed for their aggregating activity on rabbit washed platelets as described elsewhere⁶.

Platelet aggregation was induced by eluates from two main areas (Fig. 1): zones 3 and 4, which had an R_F of ~0.35, similar to that of hog leukocyte PAF¹², and zone 10, with the R_F of standard arachidonic acid¹². The aggregating activity recovered from zones 3 and 4 was unaffected when 5 μ M indomethacin was added to platelets before challenge (Fig. 1). In contrast, the eluates from zone 10 were totally inactive in the presence of the cyclo-oxygenase inhibitor, indicating that the aggregation was due to arachidonic acid (Fig. 1). Activities of arachidonate and PAF were expressed in arbitrary units—1 unit induced an aggregation producing a 1% change in light transmission.

In these experiments, PAF activity with a range of 565–4,000 U ml⁻¹ was obtained. In five control experiments no PAF was found when platelets were processed without the addition of the aggregating agent (Table 1). Preincubation of platelets for 10 min with aspirin and CP/CPK before addition of the ionophore did not affect aggregation or the formation and release of PAF. Human platelets also formed PAF on stimulation with the ionophore, although in lower amounts, even considering the lower number of human platelets in the release experiments. The presence of Ca²⁺ was an absolute requirement for the release of PAF, as 2 mM EDTA suppressed it completely.

PAF purified on TLC was totally inactivated by porcine pancreas phospholipase A₂ (Boehringer)⁵, an enzyme which specifically hydrolyses the fatty acid in the 2 position of glycerophospholipids. Furthermore, in two experiments TLC-purified PAF from rabbit platelets exhibited a similar retention time to that of PAF from hog leukocytes, when re-chromatographed using high-pressure liquid chromatography (HPLC). Therefore, PAF obtained from rabbit platelets fulfilled the criteria necessary to differentiate it from other potential aggregating agents^{5,12}: refractoriness to indomethacin and to CP/CPK, sensitivity to phospholipase A₂, solubility in organic solvents and typical migration pattern on silicic acid TLC and HPLC. PAF from human platelets was also insensitive to indomethacin and CP/CPK, was fully destroyed by phospho-

lipase A₂ and exhibited the same migration pattern as PAF from rabbit platelets or hog leukocytes on TLC (not tested on HPLC).

In seven experiments, rabbit platelets were centrifuged for 10 min after ionophore-induced aggregation. Sonication and exposure to basic pH were omitted. The recovery of $63 \pm 29\%$ of total PAF activity in the supernatants indicated that PAF was released by aggregating platelets into the extracellular medium. The overall release of PAF by the ionophore-stimulated platelets was very high. Indeed, the amount of PAF released from rabbit platelets was 10–50 times greater than that required to aggregate 100% of a physiological platelet concentration mimicked in an aggregometer tube. PAF released from platelets may thus induce aggregation and secretion of the majority of platelets in the immediate vicinity, a basic mechanism for the formation of the platelet plug during haemostasis.

Our data indicate the existence of a calcium-dependent platelet mechanism leading to the formation and release of PAF during aggregation. This release occurs equally well whether the ADP and TXA₂-dependent mechanisms are activated or whether they are inhibited by a combination of aspirin and CP/CPK. This strongly suggests that PAF is the mediator of the putative third pathway of platelet aggregation.

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Sodium-dependent cysteine transport in human red blood cells

ADEQUATE membrane transport of L-cysteine is necessary for red blood cell (RBC) survival, as this amino acid is essential for glutathione (GSH) synthesis and thus cell protection against oxidative damage. Thus, in sheep a genetically controlled L-cysteine transport defect leads to a reduced intracellular GSH concentration and shortened cell lifespan^{1–4}. Human RBCs do not have the same amino acid transport system as sheep red blood cells (SRBC)⁵, the principal component being a high capacity, medium affinity system with a specificity broadly directed towards large neutral amino acids, the L-system of Christensen⁶. L-cysteine and its analogue L- α -amino-n-butyrate are carried by the L-system in human RBCs, but with a low affinity⁷. Most experiments on amino acid transport in RBCs,

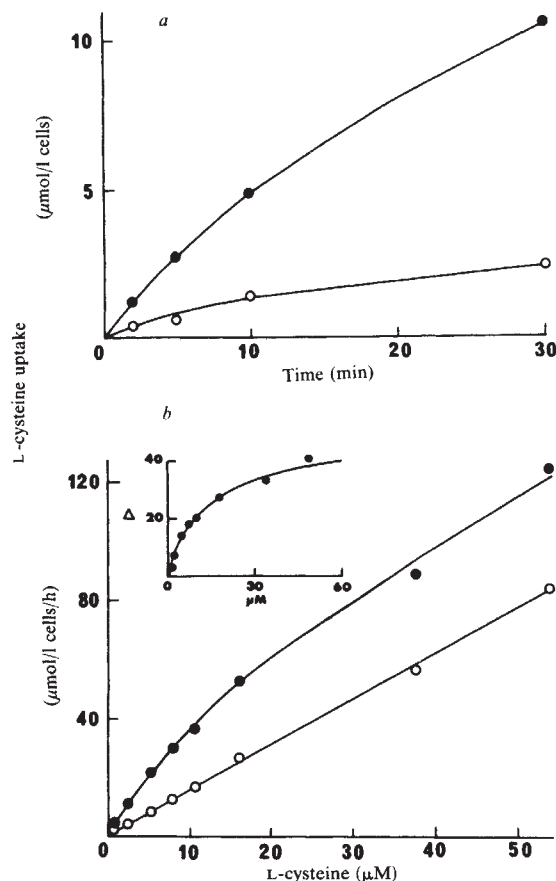


Fig. 1 *a*, Time course of L-cysteine uptake by human RBCs in sodium (●) and choline medium (○). *b*, Concentration dependence of L-cysteine uptake by human RBCs in the presence (●) and absence of sodium (○). Freshly drawn human RBCs were washed three times in medium containing 150 mM NaCl or choline Cl, 15 mM Tris-HCl (pH 7.6 at 37 °C) and 5 mM glucose. The buffy coat was discarded. L-cysteine uptake was measured at 37 °C by mixing 0.15 ml prewarmed cell suspension (haematocrit approximately 20%) in the appropriate medium with 0.15 ml prewarmed Na or choline medium containing 20 mM dithiothreitol and 1–50 μM L-cysteine (including L-U-¹⁴C-cysteine (0.5 μCi μmol⁻¹); Radiochemical Centre, Amersham, UK). Incubations were stopped at predetermined time intervals by the addition of 1 ml ice-cold magnesium chloride solution (107 mM Mg Cl₂, 10 mM Tris-HCl, pH 7.6 at 4 °C). Cells were rapidly washed four times in this medium using an Eppendorf microcentrifuge (10s, 15,000g). Finally the washed cell pellets were lysed with 0.5 ml 0.5% (v/v) Triton X-100 in water, and 0.5 ml 5% (w/v) trichloroacetic acid was added. The precipitate was removed by centrifugation, and radioactivity in the protein-free supernatants determined by β-scintillation counting with quench correction. The time course studies (*a*) were made at a final L-cysteine concentration of 10 μM. The insert curve in (*b*) is fitted as $\Delta = 49.83S/(14.2 + S)$ where Δ is the difference between Na and choline uptake rates (μmol per l cells per h) and S is the L-cysteine concentration (μM). All values are means of duplicate estimates.

particularly involving the L-system, have revealed substrate affinities with apparent K_m values in the millimolar range, much higher than the physiological plasma levels of most amino acids. We have now investigated L-cysteine uptake in human RBCs over the concentration range 1–50 μM, as human plasma levels are around 20 μM^{8,9}. As the early work of Yunis and Arimura¹⁰ has shown that glycine and L-alanine transport are partially Na-dependent, we have also investigated the effects of removing external Na on L-cysteine transport. We report here that human RBCs transport L-cysteine by a previously unidentified high affinity, low capacity, Na-dependent uptake mechanism. This system has a uniquely high affinity for its substrate and is the first Na-dependent transport mechanism to be kinetically characterised in mammalian erythrocytes. At physiological substrate

concentrations it accounts for approximately half of the L-cysteine uptake into the cell.

Figure 1a shows the time course of L-cysteine uptake (extracellular concentration 10 μM) in either Na or choline medium. Uptake in both cases was linear over the first 10 min of incubation, with the flux in choline medium threefold lower than in the presence of Na. Replacement of Na by other cations gave inhibitions of 61% choline replaced; 65% Mg replaced; 54% Li replaced and 41% K replaced, suggesting that the reduced uptake was a true Na-replacement response. Extended incubation of cells (2 h) in the presence of 10 μM L-cysteine resulted in label accumulating to 33.4 and 15.4 μmol/l cell water in Na and choline medium, respectively. Figure 1b presents the concentration dependence curve for the initial rate of L-cysteine uptake in either Na or choline medium. The flux in choline medium was linear, whereas the Na medium data were consistent with the addition of a saturable component obeying simple Michaelis-Menten kinetics with apparent K_m and V_{max} values of 14.2 μM and 49.8 μmol/l cells/h, respectively. Kinetic studies on three individuals gave apparent K_m and V_{max} values (mean ± s.e. (range)) of 17.6 ± 1.7 (14.2–20.0) μM and 53.9 ± 10.2 (38.5–73.3) μmol/l cells/h, respectively.

The possibility that white cell or platelet contamination of the RBC population was responsible for the Na-dependent L-cysteine uptake was eliminated by comparing Na-dependence before and after filtration to remove white cells and platelets¹¹, when fluxes were unaffected. The presence of reticulocytes was monitored by brilliant cresyl blue staining¹². Reticulocyte counts were in the range 0–1.4% for the three subjects used, and there was no correlation between fluxes and reticulocyte counts. The magnitude of L-cysteine uptake over extended time periods is an additional argument against this effect resulting from L-cysteine accumulation in a minor component of the total cell population. The fact that the label accumulated to a level three times greater inside the cells suggests a possible concentrative effect. However, intracellular L-cysteine metabolism may be considerable, and makes it impossible to directly relate isotope accumulation to free intracellular L-cysteine levels. Further experiments using L-alanine¹⁴, an amino acid which is not significantly metabolised in RBC, revealed a Na-dependent uptake, confirming the observations of Yunis and Arimura¹⁰, and L-alanine was found to be an effective inhibitor of Na-dependent L-cysteine uptake. Therefore, as these two amino acids seem to share the transport system, L-alanine may prove a more convenient substrate for characterising this system in more detail. Further experiments measuring Na-dependent alanine uptake in human RBCs separated according to density on a Percoll gradient¹⁵ gave 56% Na-sensitive alanine uptake (alanine concentration 0.2 mM) in the young cell fraction, and 43% in the densest fraction, corresponding to the old cells.

Increasing intracellular Na using the nystatin method¹⁶ from 14 to 95 mmol/l cell water, decreased the Na-dependent uptake by 50% (external Na 150 mM). Neither ouabain 1 mM nor furosemide 1 mM inhibited Na-dependent amino acid uptake. At an alanine concentration of 0.5 or 1 mM, it was also possible to demonstrate an amino acid-dependent Na uptake, the mean Na/alanine coupling ratio being 1.15 ± 0.06 (ref. 4).

The present results suggest that normal human RBCs possess a high affinity, low capacity Na-dependent L-cysteine transport system. The kinetic parameters of this system relate well to free L-cysteine plasma levels^{8,9}, and intracellular L-cysteine requirements for GSH biosynthesis derived from consideration of intracellular GSH turnover¹³. We therefore conclude that this transport system may represent a major pathway for L-cysteine entry into human RBCs *in vivo*.

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Ca-stimulated ATPase in brush border and basolateral membranes of rat duodenum with high affinity sites for Ca ions

THE small intestine plays an important part in the calcium homeostasis of the body, but the mechanism by which calcium is absorbed is poorly understood. In young rats, active calcium transport occurs primarily in the duodenum and is strongly dependent on the vitamin D status of the animal^{1,2}. In brush border membranes (BBM) as well as in basolateral membranes (BLM) the presence of Ca²⁺-ATPase activity has been reported^{3–5}. Moreover, a good correlation is found between Ca²⁺-ATPase activity and net calcium transport in different segments of rat small intestine⁶. These observations suggest a role for Ca²⁺-ATPase in calcium absorption. The electrochemical potential of Ca²⁺ in intestinal cells suggests that Ca²⁺ influx is passive, whereas extrusion of Ca²⁺ must be active⁶. Inherent in a role for Ca²⁺-ATPase in calcium transport is the requirement that Ca²⁺-ATPase be stimulated by concentrations occurring in the cytosol ($\leq 10^{-5}$ M). So far, Ca²⁺-ATPase activities in small intestine have been assayed in the presence of 2 to 40 mM Ca²⁺ (refs 3–5). We report here that Ca²⁺-ATPases in BBM and BLM of rat duodenum have K_m values for Ca²⁺ activation of 1.1 and 0.5 μ M respectively. Also Ca²⁺-ATPase activity below 5 μ M Ca²⁺ is higher in BLM than in BBM fragments. This asymmetrical distribution of Ca²⁺-ATPase activity may provide a mechanism for net calcium transport from lumen to blood.

Table 1 Ca²⁺-ATPase activities of BBM and BLM fragments at various Ca²⁺ concentrations

[Ca ²⁺] (μ M)	Specific activity/purification factor (μ M P _i per h per mg protein)	
	BBM	BLM
0.5	0.043 $\pm$ 0.010	0.12 $\pm$ 0.02
1.0	0.091 $\pm$ 0.023	0.17 $\pm$ 0.01
5.0	0.23 $\pm$ 0.02	0.18 $\pm$ 0.04
100.0	1.28 $\pm$ 0.03	0.42 $\pm$ 0.13

Observed specific activities (mean $\pm$ s.e.m., $n = 5$) were divided by the purification factors of the BBM or BLM respectively. The purification factor for (Na⁺ + K⁺)ATPase, a BLM marker is 7.9 ± 1.1 ($n = 5$), while for sucrase, a BBM marker is 39.8 ± 2.7 ($n = 5$). These purifications are comparable to previously published studies^{5,6}.

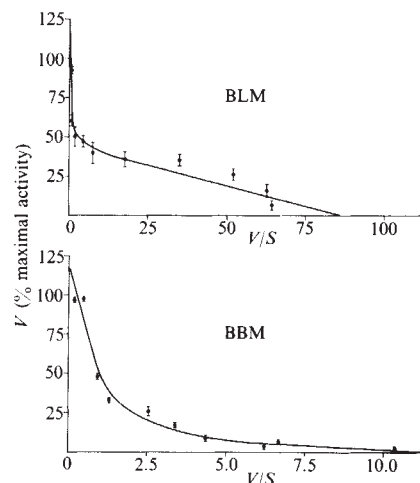


Fig. 1 Eadie-Hofstee plots of Ca²⁺-ATPase activity at various calcium concentrations. Enzymatic activity is expressed as percentage of maximal Ca²⁺-ATPase activity, for example 3.6 and 54.4 μ M P_i per h per mg protein for BLM and BBM respectively. Calcium concentrations are expressed in μ M. The data have been fitted based on a general rate equation for a two-site transport model¹⁷ by means of an iterative procedure (least-squares method). Extrapolated V_{max} values for the high affinity sites are 1.58 and 6.53 μ M P_i per h per mg protein in BLM and BBM respectively. These V_{max} values represent 45% and 12% of the total Ca²⁺-ATPase activity at 1 mM Ca in BLM and BBM fragments. K_m values for the high affinity sites are 0.5 and 1.1 μ M in BLM and BBM. K_m values for the low affinity sites are 50 and 70 μ M for BLM and BBM respectively.

BLM and BBM were isolated from the first 15 cm of the small intestine of male Wistar rats (140–160 g) using the method described by Mircheff and Wright^{5,7}. Plasma membrane fractions were essentially free of mitochondria, as succinic dehydrogenase (SDH) activity was $\leq 0.2\%$ of the initial activity. This is important as calcium inhibits ATP hydrolysis in fractions enriched in SDH activity⁵. Ca²⁺-ATPase was assayed as the difference between the rates of ATP hydrolysis in the presence and absence of calcium. The standard assay medium contained: 100 mM NaCl, 5 mM MgCl₂, 3 mM ATP and 50 mM Tris-maleate buffer (pH 7.4). Graded amounts of calcium were added. Below 50 μ M Ca-EGTA buffers were used. Free Ca²⁺ concentrations were calculated by using stability constants and equations given in the literature^{8,9}. All solutions used in Ca²⁺-ATPase assays were prepared with water which had been distilled three times in the presence of EDTA. Ca²⁺-free controls contained 4 mM EGTA. Aliquots of 25 μ l BLM or BBM suspensions (5–15 μ g protein) were incubated at 25 °C in 0.5 ml assay medium. The reaction was stopped with 0.5 ml 10% trichloroacetic acid and inorganic phosphate was assayed as described elsewhere¹⁰. Protein, sucrase, (Na⁺ + K⁺)ATPase and SDH were assayed as described previously^{5,7}.

The kinetics of calcium stimulation were studied in the concentration range 10^{-7} to 10^{-4} M and the results are presented as Eadie-Hofstee plots in Fig. 1. The plots show that the Ca²⁺-ATPase of each plasma membrane fraction has two sites, one of high and one of low affinity for Ca²⁺ ions. The K_m values for the high affinity sites are 0.5 and 1.1 μ M for BLM and BBM respectively. The K_m values are similar to those of Ca²⁺-ATPase in red cell membranes and sarcoplasmic reticulum^{11,12}. The low affinity sites have K_m values of 50 and 70 μ M for BLM and BBM respectively. Optimal activity occurs at Ca concentrations of 0.1 to 0.2 mM and no further stimulation or inhibition is observed at up to 2 mM. As the intracellular Ca concentration is expected to be lower than 10 μ M, the high affinity sites are of physiological importance. The demonstration of these sites makes the Ca²⁺-ATPase of plasma membranes of rat duodenum a candidate for a calcium pumping mechanism, in analogy to red cell membrane and sarcoplasmic reticulum calcium pumps^{11,12}.

In young rats, calcium is actively absorbed in the duodenum and the distribution of Ca^{2+} -ATPase activity among the brush border and the basolateral membranes may provide a mechanism for calcium transport⁶. As BBM and BLM are not purified to the same extent, a direct comparison of specific activities is not informative. Therefore, in Table 1 specific Ca^{2+} -ATPase activities in BBM and BLM are given after correction for their purification factors. The table shows that below $5 \mu\text{M}$ there is excess Ca^{2+} -ATPase activity in basolateral membranes. As vitamin D alters the permeability of the brush border membrane to calcium^{13,14}, an increase in Ca^{2+} -influx may result in excess Ca^{2+} extrusion across the basolateral membrane due to the excess Ca^{2+} -ATPase activity. In order to test this hypothesis, studies have to be done in which Ca^{2+} fluxes across BBM and BLM vesicles can be correlated with the amount of ATP hydrolysed by Ca^{2+} -ATPase. Unfortunately, resealed BBM vesicles are orientated right-side out with their calcium and ATP binding sites on the inside¹⁵. The currently available techniques for BLM isolation yield vesicles too leaky to permit ion transport studies¹⁶.

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Vanadate blocks cyclic AMP-induced stimulation of sodium and water transport in amphibian epithelia

INTEREST in the biological effects of vanadium has grown particularly since Cantley *et al.*¹ identified vanadate as a potent inhibitor of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ and Balfour *et al.*² reported its striking natriuretic and diuretic properties in the rat. Such findings prompted us to investigate the effects of vanadate on amphibian epithelia, a model widely used for studying, *in vitro*, the membrane transport of sodium and water^{3,4}. We found a ouabain-like action of metavanadate on active sodium transport. In addition, we report here a new biological effect of this compound on living cells: the inhibition of cyclic AMP-induced stimulation of transepithelial water flow.

The inhibition of sodium transport was mainly studied in frog skin, but similar results were obtained in toad bladder. Short circuit current (SCC) was taken as a measure of net sodium flux^{4,5}. Addition of sodium metavanadate, NaVO_3 , with V in the

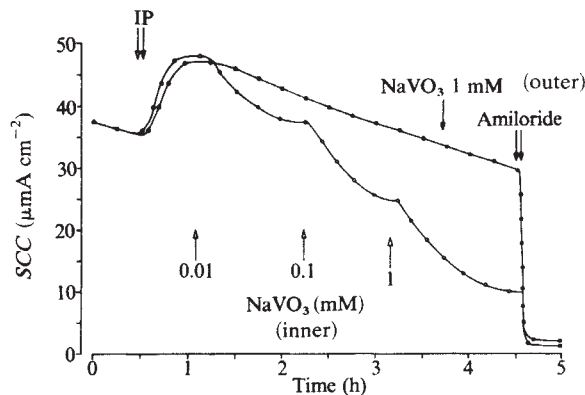


Fig. 1 Side-specific action of NaVO_3 on sodium transport across the ventral skin of the frog *R. ridibunda*. The membranes were mounted in double chambers of the Ussing type and bathed by normal Ringer solution on both sides. The two lines indicated by solid circles and open circles correspond to the SCC values of two adjacent segments of the same skin. Exposure of both segments to a β -adrenergic agonist (isoprenaline, IP, $1 \mu\text{M}$) induced similar increments in SCC. Subsequent addition of NaVO_3 to the inner solution resulted in a progressive, ouabain-like, dose-dependent fall in SCC; in contrast, addition of NaVO_3 to the outer solution had no effect. The two skin segments were sensitive to amiloride (10^{-4} M), a specific blocker of the sodium entry sites of the outer surface of the epithelium; this indicates the sodium nature of the SCC in this epithelium.

+5 oxidised state, to the solution bathing the internal side of the epithelium, resulted in a dose-dependent decrease in SCC (data not shown). The degree of inhibition and its time course were very similar to those observed in the presence of ouabain or harmaline⁶, two well known inhibitors of the sodium pump. A similar fall in SCC was seen in skins with higher rates of sodium transport due to pre-exposure of the epithelium to natriuretic agents such as catecholamines (Fig. 1), neurohypophyseal hormones, cyclic AMP and theophylline. In addition, pre-exposure of the skins to NaVO_3 (10^{-3} M), almost completely prevented the stimulation of SCC by any of the above agents (Table 1). Finally, vanadate was clearly demonstrated to act at only one side of the membrane by the lack of effect of this compound when added to the solution bathing the outer surface of the skin (Fig. 1).

An inhibition of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ of frog skin by NaVO_3 is the simplest hypothesis to explain the changes in SCC described here³. The concentrations of vanadate required to reduce SCC were rather high, but the same applies to ouabain and harmaline, in this tissue⁶. Such relative refractoriness of the enzyme may be related to the presence of an endogenous, ouabain-like inhibitor of the sodium pump, as recently suggested⁷, and/or to an intracellular site of action of vanadate⁸ and harmaline⁶.

As the inactivation of the sodium pump affects the last step of the transepithelial movement of sodium, other biological effects of vanadium could remain undisclosed, for example, an interaction between vanadate and the cyclic AMP system of the epithelial cell. We previously observed this type of situation while studying the effects of harmaline in amphibian skin⁶. Therefore, we looked for effects of NaVO_3 on another transport system, that of water. The osmotic flow ($J_{\text{H}_2\text{O}}$) across the bladder or the skin of toads, *Bufo marinus*, was monitored continuously by means of an automatic, volumetric technique^{6,9,10}. Addition of NaVO_3 , up to 10^{-3} M , to the internal medium, did not cause any marked change in $J_{\text{H}_2\text{O}}$ although a transient but reproducible stimulation was often noted during the first 3 min, particularly in toad bladder. A conspicuous effect was seen, however, when the epithelia were subsequently challenged with a hydrosomotic agent, such as vasopressin, in toad bladder (Table 1) and vasopressin or isoprenaline in toad skin (data not shown).

The increase in J_{H_2O} induced by these agents was inhibited by vanadate in a dose-dependent manner at concentrations of 10^{-5} – 10^{-3} M. Similar results were obtained with theophylline (Table 1). Taken together, these effects of vanadate on J_{H_2O} could be interpreted as evidence for an inactivation of adenylyl cyclase. If this were the case, one would expect no inhibition of the hydrosmotic effect of exogenous cyclic AMP by $NaVO_3$ (ref. 3). The experimental results, however, revealed completely the opposite (Table 1). Vanadate also inhibited J_{H_2O} in epithelia previously exposed to a hydrosmotic agent and thus having a high rate of water flow before $NaVO_3$ addition; interestingly, the fall in J_{H_2O} did not go beyond basal water flow (Fig. 2). Finally, as already seen with SCC, the vanadate effect on J_{H_2O} was side-specific; when added to the outer medium, $NaVO_3$ did not affect either basal or stimulated J_{H_2O} .

The biological effects of vanadate on both sodium and water transport described here are relevant to recent physiological^{2,11} and biochemical^{1,8,12–15} observations made with vanadium. The effects on SCC are consistent with the natriuretic properties² and the inhibition of $(Na^+ + K^+)ATPase$ ^{1,8,12–14} reported by others. The effects on J_{H_2O} do not seem to be directly related to the sodium pump inhibition. It has been found in this and other laboratories that ouabain has no appreciable effect on J_{H_2O} in toad bladder, despite near-maximal reductions of SCC¹⁶. On the other hand, an interaction between vanadate and the cyclic AMP system would easily explain the J_{H_2O} data shown in Fig. 2

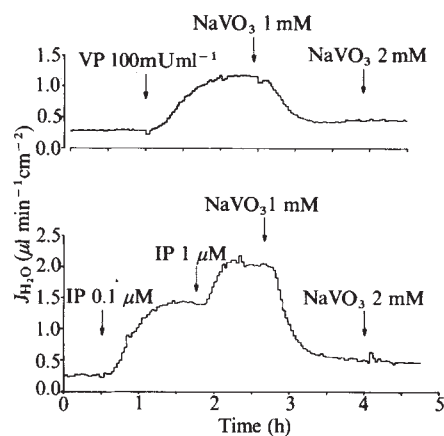


Fig. 2 Two examples of the effect of $NaVO_3$ on cyclic AMP-induced stimulation of water transport (J_{H_2O}) across the ventral skin of toads *B. marinus*. The hydrosmotic actions of both vasopressin, VP (upper half), and isoprenaline, IP (lower half), were almost completely blocked by $NaVO_3$ added to the inner solution. The automatic technique used to record J_{H_2O} has been described elsewhere^{6,9}. The solutions bathing the epithelium were: normal Ringer (inner surface) and Ringer diluted 10-fold (outer surface). Although vanadate inhibited both basal and stimulated SCC, this agent seems to block only the cyclic AMP-induced stimulation of J_{H_2O} but not basal J_{H_2O} .

Table 1 Block by vanadate of stimulated SCC and water flow in amphibian epithelia

	a, SCC (frog skin) ($\mu A\ cm^{-2}$)			
	Control		$NaVO_3$ (1 mM)	
	Basal	Peak	Basal	Peak
Oxytocin (10)	17.5	39.0*	6.1	9.1*
50 mU ml ⁻¹	±3.2	±4.3	±0.9	±1.0
Isoprenaline (10)	15.6	46.6*	6.7	9.0‡
1 μM	±2.4	±4.2	±0.8	±1.0
Theophylline (9)	19.1	66.8*	5.8	12.7†
10 mM	±3.6	±6.9	±0.8	±1.7
Cyclic AMP (8)	12.7	20.1*	6.3	7.4‡
5 mM	±1.9	±2.0	±1.2	±1.1
	b, J_{H_2O} (toad bladder) ($\mu l\ h^{-1}\ cm^{-2}$)			
	Control		$NaVO_3$ (1 mM)	
	Before	After	Before	After
Vasopressin (9)	1.6	114.0*	5.3	15.6*
50 mU ml ⁻¹	±0.6	±14.8	±1.3	2.7
Theophylline (6)	1.8	71.2*	3.6	5.4
10 mM	±0.4	±7.3	±1.3	±1.7 (NS)
Cyclic AMP (6)	7.3	48.5*	4.5	5.6
5 mM	±1.3	±5.9	±1.8	±2.5 (NS)

a, Abdominal skins of frogs *Rana ridibunda* were challenged with different natriuretic agents. In each experiment two segments of the same skin were used, one of which had been pre-exposed to $NaVO_3$ added to the internal solution. Values in the first and third columns correspond to SCC recordings before addition of the agent indicated on the left; values on second and fourth columns correspond to peak responses obtained after exposure to the same agent. b, A similar protocol was applied to quarter-bladders of toads *Bufo marinus* to determine the effect of vanadate on cyclic AMP-dependent stimulation of water flow (J_{H_2O}). Values represent cumulative J_{H_2O} (for 60-min periods), before and after addition of the hydrosmotic agent indicated on the left. The first two columns show results using the control quarter-bladders, the last two, paired quarter-bladders exposed to vanadate. Similar results were obtained in toad skin. Only the skins were challenged with isoprenaline, as the urinary bladder lacks β -adrenergic receptors. All values are mean $\pm$ s.e.m. The number of paired studies is indicated in parentheses. Statistical analysis was done by using the paired *t*-test. Significance of *P* values is indicated by NS (non-significant), * <0.001 , † <0.01 and ‡ <0.05 . On exposure to $NaVO_3$, fractional increases in SCC and cumulative J_{H_2O} values were significantly different with respect to control values for all the natriuretic and hydrosmotic agents tested.

and Table 1. Without obviously excluding an effect of vanadate on adenylyl cyclase¹⁵, a site of action beyond cyclic AMP generation must be postulated to account for the inhibition of the effect of exogenous cyclic AMP. Two lines of evidence suggest that an ATPase linked to mechanochemical proteins might be such a target: (1) the involvement of microtubules and microfilaments in epithelial water transport^{17–19} and (2) the inhibition of dynein-ATPase by vanadate^{20–22}. Further work is needed to elucidate this mechanism, which possibly contributes to the huge diuresis observed in the rat. Overall, our results indicate that the amphibian epithelium is a useful model for distinguishing between the many biological actions of vanadium.

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Neuronal localisation of immunoreactive enkephalin and β -endorphin in the earthworm

SINCE the report by Simantov *et al.*¹ invertebrates have been thought to lack endogenous opiates and opiate receptors. As we have previously observed peptidergic neurones containing immunoreactive pancreatic polypeptide or vasoactive intestinal peptide in the earthworm², we decided to search for, and report here our finding of, nerves storing immunoreactive enkephalin or β -endorphin in this species.

Various segments of earthworms (*Lumbricus terrestris*) were cut and frozen in a propane-propylene mixture cooled to the temperature of liquid nitrogen. After freeze-drying the specimens were fixed in formaldehyde vapour at 80 °C and embedded in paraffin *in vacuo*. Sections, 5 μ m thick, were deparaffinised and used for the immunohistochemical demonstration of enkephalin or β -endorphin, using either the indirect immunofluorescence technique³ or the PAP technique of Sternberger⁴. The enkephalin antiserum used was raised against synthetic Leu-enkephalin coupled to bovine serum albumin. The characteristics of this antiserum are described elsewhere^{5,6}. The β -endorphin antiserum (Milab) was raised as follows. Synthetic human β -endorphin (β -lipotropin (61–91); Peninsula) was emulsified in Freund's complete adjuvant. It was injected intracutaneously at multiple sites in rabbits, at a dose of 100 μ g of β -endorphin per rabbit. Two booster injections of 20 μ g were given at 3-monthly intervals. The third booster injection of 100 μ g β -endorphin was given a further 3 months later, and sera

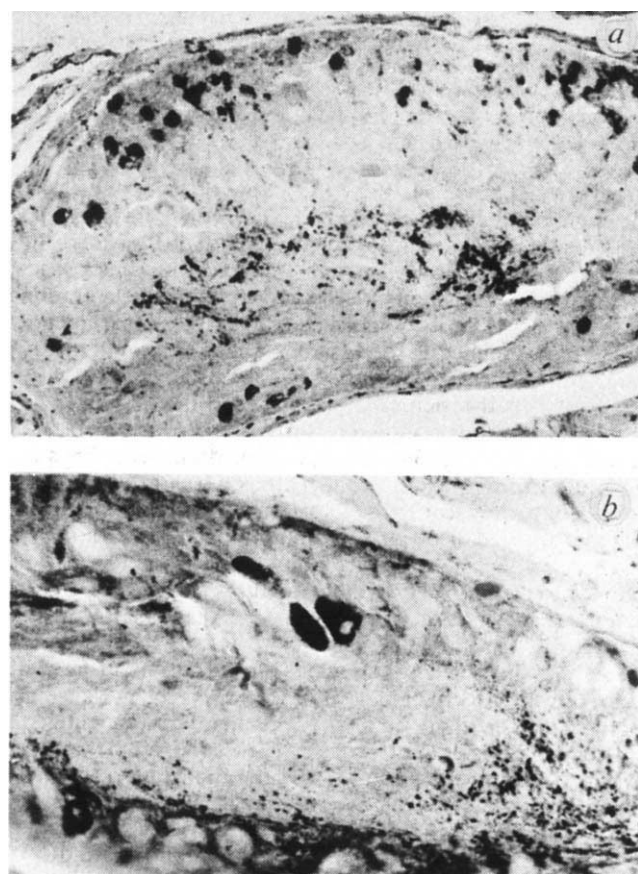


Fig. 1 Cerebral ganglion of the earthworm. Circumpharyngeal connective to the right. *a*, Many Leu-enkephalin-immunoreactive nerves and cell bodies (PAP staining). Note the peripheral localisation of the cell bodies and the central localisation of the nerves. $\times 300$. *b*, β -Endorphin-immunoreactive nerves and cell bodies. $\times 400$.

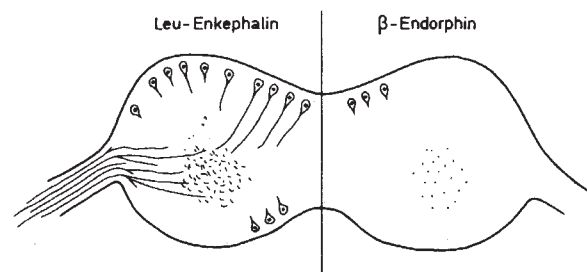


Fig. 2 Cross-section of the cerebral ganglion of the earthworm to show the distribution and relative frequency of enkephalin- and β -endorphin-immunoreactive nerves and cell bodies. Some of the nerves seem to reach the circumpharyngeal connectives. Transsected nerves are indicated by dots.

were collected 2 months after this. The specificity of the β -endorphin antiserum (diluted 1:10,000) was tested in a radioimmunoassay system. It did not cross-react ($<0.1\%$) with α -MSH (ACTH (1–13)), β -MSH (β -lipotropin (41–58)), Met-enkephalin (β -lipotropin (61–65)), α -endorphin (β -lipotropin (61–76)), γ -endorphin (β -lipotropin (61–77)), β -lipotropin (60–65), β -lipotropin (39–45), ACTH (1–24), ACTH (34–39) or ACTH (4–11).

The enkephalin antiserum was used in a dilution of 1:40 (immunofluorescence) or 1:400 (PAP staining), and the β -endorphin antiserum in a dilution of 1:20 or 1:200, respectively. Controls included application of antiserum inactivated by addition of excess Leu-enkephalin or β -endorphin (Peninsula) (10 μ g per ml diluted antiserum). Many neuronal cell bodies of different sizes and nerve fibres displaying intense enkephalin immunoreactivity were found in the cerebral ganglion. Characteristically, the cell bodies were located in the periphery whereas the nerve fibres occurred in the central part (Figs 1*a*, 2). Immunoreactive nerves were numerous within the cerebral ganglion and extended into the circumpharyngeal connectives. Immunoreactive nerve cell bodies and a few nerves were occasionally found in the subpharyngeal ganglion and in the abdominal ganglia. Cell bodies displaying β -endorphin immunoreactivity had a distribution similar to that of the enkephalin immunoreactive ones. Generally, the β -endorphin cells seemed to be larger in size and much fewer in number (Figs 1*b*, 2). They were clearly distinct from the cell bodies containing immunoreactive enkephalin. β -Endorphin-immunoreactive nerves were scattered in the central part of the cerebral ganglion but were absent from the circumpharyngeal connectives. In the subpharyngeal ganglion and the abdominal ganglia immunoreactive cell bodies and nerves were rare.

The results obtained indicate that enkephalin and β -endorphin, or closely related peptides, exist in the neurones of the earthworm. Hence, this suggests that opiate receptors may also be found in this species. In view of the present findings the belief that endogenous opiates and opiate receptors do not exist in invertebrates may have to be reconsidered.

While this manuscript was being prepared Rémy and Dubois⁷ described a few α -endorphin-immunoreactive cell bodies in the subpharyngeal ganglion of the earthworm. They did not find β -endorphin immunoreactivity.

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Thermal activation of the visual transduction mechanism in retinal rods

INVERTEBRATE photoreceptors show electrical changes which apparently result from isomerisation of single rhodopsin molecules by light or thermal energy^{1–3}. Observation of corresponding phenomena in vertebrates has been prevented by intercellular electrical coupling, which averages membrane potential over many photoreceptors^{4–6}. Recently, however, recordings of membrane current from individual rod outer segments have revealed responses to single photons^{7,8}. Here we report that similar electrical events occasionally occur in darkness, perhaps because of thermal isomerisation of rhodopsin.

Membrane current was recorded from single rod outer segments in toad (*Bufo marinus*) retina using a method described previously⁹. Pieces of thoroughly dark-adapted retina were

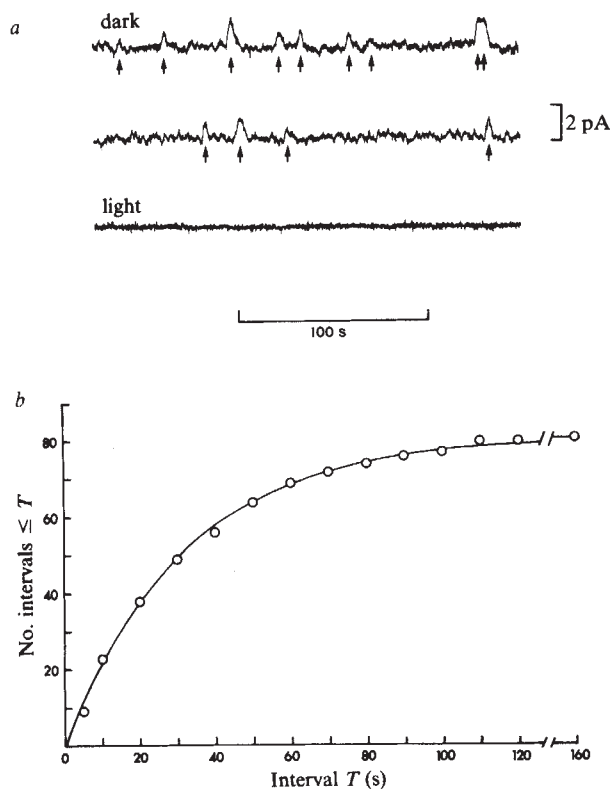


Fig. 1 *a*, Sample records of rod outer segment current in darkness (two upper traces, sequential records) and bright light (bottom trace). Arrows below traces indicate times at which current exceeded a criterion level 0.5 pA above the baseline; these times are taken as occurrences of discrete events, discussed in the text. Using this method a total of 82 events were counted over a period of 2,631 s. In light the photocurrent reached a saturating amplitude of 19 pA and no events were evident in the observation period of 1,106 s. Records were low-pass filtered at 5 Hz with a 6-pole filter. Temperature 22 °C. *b*, Cumulative distribution of intervals between successive events in the same cell shown in *a*. Circles are experimental points; curve drawn according to equation (1) in text with $\tau = 32$ s, $N = 81$.

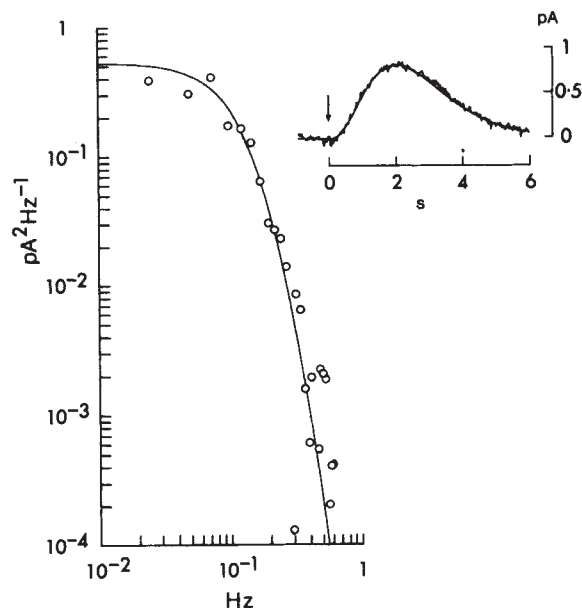


Fig. 2 Comparison of power spectral densities of dark events and single photon response in a rod. $\circ$, Difference power spectrum of dark events obtained as spectrum of all dark sweeps minus spectrum of dark sweeps not containing events. Experimental spectra based on 43 sweeps, each 40.96 s long, digitised at 20-ms intervals after low-pass filtering at 15 Hz. Continuous spectrum calculated from the Fourier transform of equation (2) in the text, with $\alpha = 1.33$ s⁻¹. Vertical scaling of theoretical spectrum, chosen to give best fit to points, corresponds to a mean rate of 0.022 s⁻¹ assuming dark events had the same amplitude (1 pA) as this cell's single photon response. Event frequency determined by counting was 0.023 s⁻¹. Temperature 21.6 °C. Inset, jagged curve is the average of 80 responses of the same cell to dim flashes delivering an average of 0.8 effectively absorbed photons per flash. Flash timing shown by arrow. Smooth curve is equation (2), with $\alpha = 1.33$ s⁻¹.

placed in a chamber containing oxygenated Ringer and viewed in a compound inverted microscope. Retinal isolation and subsequent procedures were carried out under infrared illumination with an IR/visible image converter. Under visual control the outer segment of a rod in one of the pieces of retina was drawn by gentle suction into the tip of a glass micropipette. A low-noise amplifier connected to the inside of the pipette gave an output voltage proportional to membrane current. This current signal, the output of a light stimulus monitor, and synchronising pulses were led to an FM tape recorder for later analysis in a PDP 11/34 computer. For 'dark' recordings the preparation was enclosed in a black box which attenuated the very dim light (low scotopic level for humans) in the experimental room by a factor of 10⁶. Heated or cooled water could be circulated through a jacket surrounding the central Ringer pool in the experimental chamber; temperatures were measured with a calibrated thermistor about 0.5 mm from the tip of the recording electrode.

Figure 1a shows records of outer segment current in darkness (upper traces) and bright light (bottom trace). The noise in light was of instrumental origin, and the dominant component had a magnitude predicted by the measured value of the leakage resistance between electrode wall and outer segment membrane. The excess fluctuation in the dark records consisted of two components: (1) occasional discrete events (indicated by arrows below dark traces) of about 1 pA amplitude, and (2) a continuous small-amplitude fluctuation, not discussed further here. The discrete events resembled the average response to a single photon and in this cell were estimated to occur at a mean rate of 0.031 s⁻¹. In the experimental conditions, however, the expected rate of photoisomerisations from stray light was at least two orders of magnitude below the observed event rate. This suggests that the events were not triggered by photons

but reflected spontaneous activation of the transduction mechanism.

If random independent excitations occurred in the rhodopsin molecules or intracellular disks of the outer segment the intervals between events should be exponentially distributed. Figure 1b compares an experimental interval histogram with this prediction. The continuous curve was drawn according to

$$n = N(1 - \exp(-T/\tau)) \quad (1)$$

where n is the number of intervals less than or equal to T , τ is the average interval, and N is the total number of intervals observed. The satisfactory agreement between experimental results and equation (1) supports the interpretation that the occurrence of events is a Poisson process describable by the single parameter, τ , the average interval between events. Similar agreement between experimental results and theoretical curves was observed in seven other experiments, with a range of values for τ of 76–17 s at temperatures of 15–26 °C.

The amplitude of the dark events was similar to the amplitude of the same cell's single photon response. To compare the kinetics of dark events and single photon responses we examined their power spectra; previous work⁸ has shown that the current noise evoked by dim steady light has the spectrum of the average single photon response. The spectrum of the dark events was obtained as the spectrum of all dark sweeps minus the spectrum of dark sweeps not containing events. The average single photon response in each experiment was fitted by the relation⁹ (Fig. 2 inset)

$$r(t) = k(\alpha t)^3 e^{-\alpha t} \quad (2)$$

where r is the photocurrent as a function of time t after photon absorption, k is a scaling constant and α is a rate constant characteristic of the cell. This equation would apply if photoisomerisations cause electrical action by a process involving a series of four first-order delays^{10,11}. The points in Fig. 2 are the spectrum of the dark events, and the curve is the calculated spectrum of the same cell's single photon response. Similarly close agreement between observed and calculated spectra was seen in experiments on eight other cells. The ability of the equation for the flash response to predict the spectrum of the dark events suggests that they are triggered by fluctuations at the input of the cascade. A simple explanation would be that the dark events result from a thermal configuration change in rhodopsin itself.

Figure 3 shows the temperature dependence of the rate of occurrence of dark events. The slope of the Arrhenius plot gives an activation energy E_a of 20.9 kcal mol⁻¹ for the thermal excitation process. In similar experiments on five cells the value of E_a was 23.2 ± 3.2 kcal mol⁻¹ (mean ± s.d.). The mean frequency of events in these experiments was 0.021 s⁻¹ at 20 °C.

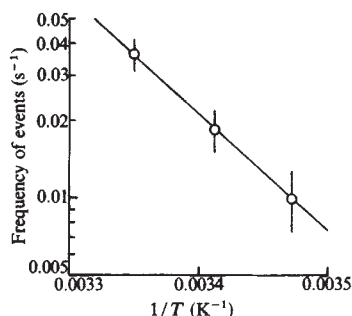


Fig. 3 Arrhenius plot of frequency of occurrence of dark events in a rod (log scale) against reciprocal absolute temperature. Detection of events in the continuous background noise was facilitated by digitally processing the current records with a 'matched filter' (ref. 15). In this procedure equation (2) was fitted to the rod's single photon response and then cross-correlated with the dark recordings. Vertical bars are based on the square root of the number of events counted. Slope of linear regression line corresponds to activation energy E_a of 20.9 kcal mol⁻¹.

Assuming that the rod outer segment contains 2×10^9 rhodopsin molecules and that thermal fluctuations in rhodopsin cause the dark events, the estimated rate constant for thermal excitation of rhodopsin is 10^{-11} s⁻¹. This corresponds to a molecular half life of the order of 1,000 yr. Additional parameters for the thermal excitation of rhodopsin at 20 °C were calculated as: Gibbs free energy of activation $\Delta G^\ddagger = 31.9 \pm 0.13$ kcal mol⁻¹, entropy of activation $\Delta S^\ddagger = -31.7 \pm 11.2$ e.u.

Ashmore and Falk¹², by analysis of membrane noise in neurones postsynaptic to dogfish rods, estimated values of rate constant and $\Delta G^\ddagger$ similar to our own but found E_a to be 36 kcal mol⁻¹ and $\Delta S^\ddagger$ to be +13 e.u. The differences may reflect different experimental methods. Chemical studies show that thermal isomerisation of 11-*cis* retinal in aqueous digitonin solution¹³ is associated with a small negative activation entropy of -12.5 e.u. and E_a of 24.5 kcal mol⁻¹, whereas for thermal denaturation of cattle rhodopsin in rod fragments Hubbard¹⁴ found $\Delta S^\ddagger = +214$ e.u. and $E_a = 100$ kcal mol⁻¹. Furthermore, extrapolation to 20 °C gives a rate constant for denaturation in rod fragments three orders of magnitude below the excitation rate constant derived here. Isomerisation is thus the more likely trigger of dark events. In a variety of solvents, however, derived rate constants for thermal isomerisation of 11-*cis* retinal alone are two to three orders of magnitude larger than the spontaneous excitation rate constant. This implies that linkage with opsin stabilises the 11-*cis* configuration of the chromophore.

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Photoinduced electron transport across phospholipid wall of liposome using methylene blue

PHOTOSENSITISED electron transport across membranes is a useful model reaction for photosynthesis. We have previously reported¹ the photoredox reaction in/on liposomes. Other similar studies have used chlorophyll as a sensitizer^{2,3}, but this compound is easily broken and difficult to purify. The surfactant analogues of metal complexes (particularly tris-(2,2'-bipyridine) Ru²⁺)^{4–6} are also widely used, but with such photosensitisers which possess long alkyl chains, electron transport does not occur in the absence of electron carriers such as quinones or carotenes. We report here the occurrence of photosensitised electron transport across the single phospholipid wall of the

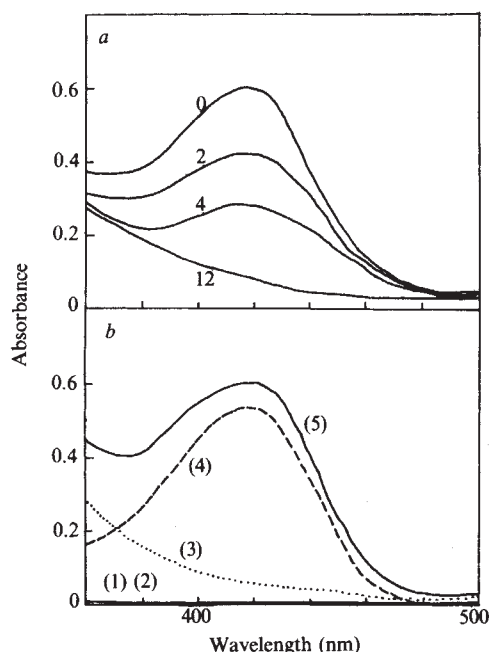


Fig. 1 a, Progressive spectral changes with time of irradiation; [MB] = 0.01 mM; [ASc] = 0.008 M. Numbers on each curve indicate the irradiation time (min.). UV spectra of each component in Tris buffer; (1) [MB] = 0.01 mM; (2) [ASc] = 0.008 M; (3) dispersed lecithin; (4) [potassium ferricyanide] = 4 mM; (5) 1 + 2 + 3 + 4.

liposome using methylene blue as both a sensitizer and an electron carrier. This is the first report of photosensitized electron transport across a membrane using a dye not only as a photosensitizer but also as an electron carrier.

The reaction was carried out in the single lamella liposome system. The liposome system, containing water-soluble electron acceptor ($K_3Fe(CN)_6$) only in the inner aqueous phase of the vesicle and water-soluble electron donor (ascorbic acid) only in the outer aqueous phase, was prepared in the following way. Lecithin (isolated from egg-yolk⁷) was dispersed in 1 M $K_3Fe(CN)_6$ solution buffered with 1 M Tris-Cl and 0.1 M KCl, pH 7.5. The dispersion was then ultrasonicated under N_2 gas and the undispersed phospholipids were removed by gel filtration over a column of Sephadex G-50 equilibrated with the buffer solution. Then a given amount of methylene blue

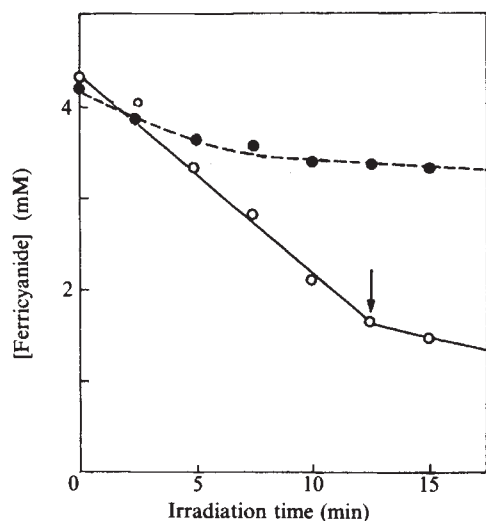


Fig. 2 Change in ferricyanide concentration with time of irradiation. ○, [MB] = 0.008 mM, [ASH] = 8 mM; [ASH] > [ferricyanide]. ●, [MB] = 0.008 mM, [ASH] = 0.08 mM; [ASH] < [ferricyanide]. Arrow indicates the time at which the light was cut off.

was added. After being degassed by passing N_2 gas, the dispersion was added to a solution of ascorbic acid at a final concentration of 8 mM. As soon as the solution was mixed, it was sealed in a Pyrex tube with a UV cell on the side arm (or sealed in a UV cell as a control). The photoreaction was carried out immediately by irradiating the solution with a super-high-pressure mercury lamp. Light of wavelengths shorter than 460 nm was completely removed by the use of a UV-46 filter.

In these conditions methylene blue was only very slightly reduced by ascorbic acid without light. However, when the light was irradiated, the absorbance at 420 nm decreased rapidly (Fig. 1a). Neither ascorbic acid nor methylene blue absorbs the light at 420 nm (Fig. 1b), and the absorption of lecithin was constant even with irradiation. Therefore, the decrease in the potassium ferricyanide concentration could be measured by measuring the decrease in the absorbance at 420 nm.

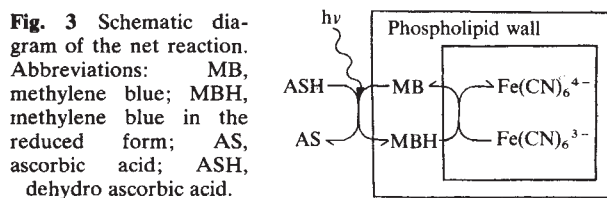
Table 1 Relative reaction rates in different conditions

	Relative reaction rates	
	Irradiated	Non-irradiated
(A) Complete system	1	0.05
(B) A – ascorbic acid	0.01	0
(C) A – methylene blue	0	0

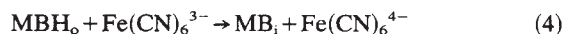
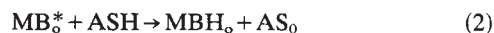
Complete system: methylene blue, 0.01 mM; ascorbic acid, 0.01 M.

Figure 2 shows the decrease in the ferricyanide concentration with time of irradiation. When the ascorbic acid concentration is higher than that of potassium ferricyanide, the reaction proceeds until all the ferricyanide is completely reduced. If the light is cut off, the reaction stops. When the ascorbic acid concentration is lower than that of potassium ferricyanide, the reaction stops after all ascorbic acid has been oxidised. These results confirm that ferricyanide is reduced.

As shown in Table 1, the reduction of $K_3Fe(CN)_6$ did not occur in the absence of methylene blue or in the absence of ascorbic acid, even when the system was irradiated. The reaction was almost undetectable without light. This shows that either direct reduction of $K_3Fe(CN)_6$ by ascorbic acid or the penetration of ascorbic acid did not occur. However, ascorbic acid cannot exist in the reduced form in the inner aqueous phase, and furthermore, the reduction rate increased with increase in the concentration of methylene blue.



From these results, the reaction can be summarised as proceeding through the following steps (see also Fig. 3).



where MB_o and MBH_o are methylene blue and reduced methylene blue in the outer aqueous phase, MB_i is methylene blue in the inner aqueous phase, AS and ASH are ascorbic acid and dehydroascorbic acid, and * indicates an excited state.

We do not understand exactly how step (3) occurs, but it is clear that the electron is transported from ascorbic acid in the outer aqueous phase to $K_3Fe(CN)_6$ in the inner aqueous phase, and that this electron transport is induced by the photo-irradiation. We are continuing our investigation of the transport process.

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Sequence divergence of rainbow trout protamine mRNAs; comparison of coding and non-coding nucleotide sequences in three protamine cDNA plasmids

SPERMATOGENESIS in the rainbow trout (*Salmo gairdnerii*) is associated with the onset of synthesis of the protamines^{1,2}, a family of small, highly basic proteins consisting of 32 or 33 residues, of which approximately two-thirds are arginine³. By displacing the histones, the protamines have a unique role in packaging sperm DNA. The biosynthesis of the protamines has been studied in some detail, and polyadenylated protamine mRNA of about 290 nucleotides has been isolated from trout testis^{4,5} and shown by *R₀t* analysis to be made up of three to four components^{5,6}. More recent work suggests that there are about six different protamine genes per haploid genome⁷. In contrast to the interspecific conservation of histone sequences, the observed rate of protamine sequence divergence between the closely related clupeid fishes such as trout, salmon and herring, is very high, and there is evidence that protamine genes have arisen following a doubling of the ancestral fish genome. Comparison of the cloned complementary DNA sequences and ultimately of the genes themselves will help to elucidate the molecular events involved in the evolution of these proteins. To study the chromosomal organisation of these genes and the control of their expression during spermatogenesis, I have constructed cDNA clones using purified poly(A)⁺ mRNA as starting material. Here, the sequences of three of these recombinants are compared, revealing an unexpected pattern of divergence in the coding and non-coding regions, as well as a mutational 'hot-spot' corresponding to the major phosphorylation site of the protamines.

The sequences of the cloned cDNA fragments were determined by the methods of Maxam and Gilbert⁸. Figure 1 is a representative sequence ladder and Fig. 2 shows the extent of the cloned sequences aligned with reference to the functional chain terminator codon TAG. pTP4 contains a complete coding sequence, as well as 5'- and 3'-non-coding regions, and pTP8 and pTP11 have incomplete coding sequences; the fact that they both start at the same point may reflect some secondary structure of the parent mRNA. Both pTP4 and pTP8 contain the hexanucleotide corresponding to A₂UA₃ which seems to be a general feature of eukaryotic poly(A)⁺ mRNAs⁹; the presence of this hexanucleotide in protamine mRNA has been reported previously¹⁰.

The coding regions of the three clones were located by reference to the published amino acid sequences for rainbow trout protamines³ (Fig. 3a, b). When these coding sequences are compared (Fig. 4) there is a striking homology between pTP8 and pTP11, with only one substitution in 80 nucleotides. The coding region of pTP4 is markedly different from that of the other two, and there is only a 78% homology when the

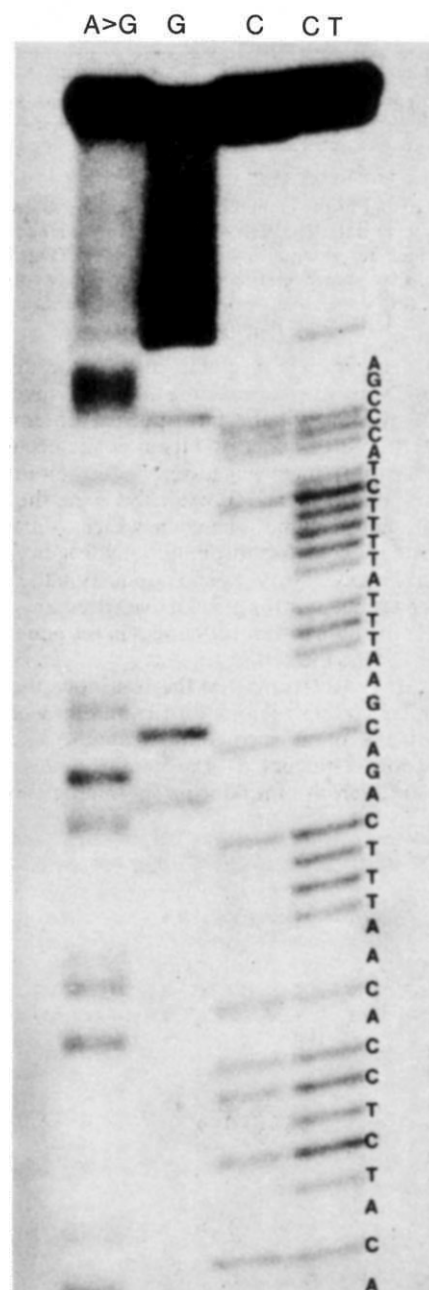


Fig. 1 Poly(A)⁺ mRNA was purified from pooled rainbow trout testes, double-stranded cDNA synthesised and inserted into the *Pst*I site of plasmid pBR322 using dG · dC homopolymer tailing, and transfected into the disabled host strain χ 1776 (ref. 5). Recombinants containing protamine cDNA sequences were identified by liquid hybridisation to cDNA synthesised from purified protamine mRNA⁵. Three recombinants, pTP4, pTP8 and pTP11, were chosen for sequence analysis. All work involving recombinant DNA was carried out in a Category II facility in accordance with GMAG recommendations. This figure shows a sequence gel of the 3'-non-coding region of pTP8. *Hap*1-cleaved pTP8 insert DNA was labelled at this cleavage site using T4 polymerase, and the unique end-labelled fragments separated on a 10% acrylamide gel and sequenced by the method of Maxam and Gilbert⁸. The gel shown is 12% acrylamide, 7 M urea.

sequences are aligned, taking into account the putative deletion in pTP4. In contrast, a comparison of the non-coding regions of these cDNA fragments shows a complete reversal of these homology relationships (Fig. 3a). Thus, from the chain termination signal to the 3'-end of the segment of pTP11 which has been cloned, pTP8 and pTP11 differ by over 20% in nucleotide sequence; over this same region pTP4 and pTP11 are identical. This is unexpected because at least where it has been

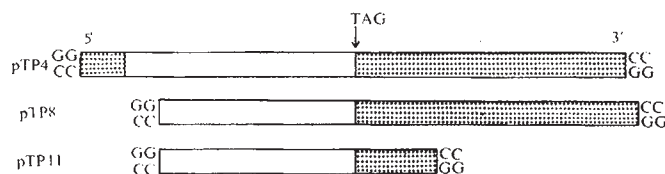


Fig. 2 The extent of the cloned cDNA fragments as determined from the sequence data. The cDNA fragments are aligned using the functional chain termination signal codon TAG (Fig. 3). Non-coding regions are shown as stippled and the polarity corresponds to that of the mRNA sequences. The cDNA fragments are flanked by dG·dC homopolymer linkers.

examined between species in globin mRNAs, coding regions are conserved whereas non-coding regions diverge more rapidly¹¹⁻¹³. In the case of the intraspecific protamine clones both this pattern of divergence and its opposite occur.

Ohno and Atkin¹⁴ have suggested a tetraploid origin of salmonid fishes, and evidence of extensive gene duplication is provided by studies of salmonid enzymes such as malate dehydrogenase¹⁵ and glucose-6-phosphate dehydrogenase¹⁶. If the protamines have evolved from a gene duplication then since this event there has been strong selective pressure favouring conservation of the non-coding sequences in the genes cloned in pTP4 and pTP11, and the coding regions have been free to drift. Alternatively, the mRNAs may be the result of splicing events linking dissimilar coding regions to a common 3'-non-coding sequence. Both explanations could implicate the 3'-non-coding region in the coordinate control of protamine genes which may be expressed at different times during spermatogenesis¹⁷.

Within the 3'-non-coding regions of pTP4 and pTP8 the longest run of conserved sequence is one of 22 bases including the hexanucleotide AATAAA. Substantial homology has been found in this region between rabbit, human and mouse β -globin nucleotide sequences^{18,19}, suggesting that these flanking sequences, as well as the AATAAA, may be involved in some common control function.

None of the predicted amino acid sequences corresponds exactly to any of those published³ (Fig. 3a, b). One explanation for this is that the cloned sequences correspond to minor variants within the protamine population, as the mRNA was isolated from pooled testes. However, a more likely explanation is that some error occurred in the amino acid sequencing. The pattern of amino acid substitutions found in the predicted sequences suggests there is a mutational hot spot in residues 6-11 of the N-terminal region (where the N-terminal methionine residue of pTP4 = residue 1), as five out of six residues have changed in this region by base transition and sequence rearrangement. This hot spot is present to a lesser extent in the clupeines^{20,21} but is not seen in the sequences reported by Ando and Watanabe³. It may be significant that this region contains three of the four serine residues phosphorylated by cyclic AMP-dependent protein kinase during histone displacement and nucleoprotamine assembly^{22,23}.

The high frequency of arginine and serine in the protamines makes their mRNAs interesting for the study of codon usage. Davies *et al.*²⁴ reported that there is no preferential use of any arginine codon in protamine mRNA and that the dinucleotide CpG is under-represented, as in vertebrate DNA²⁵ and mRNA²⁶, despite the fact that it is present in four of the six

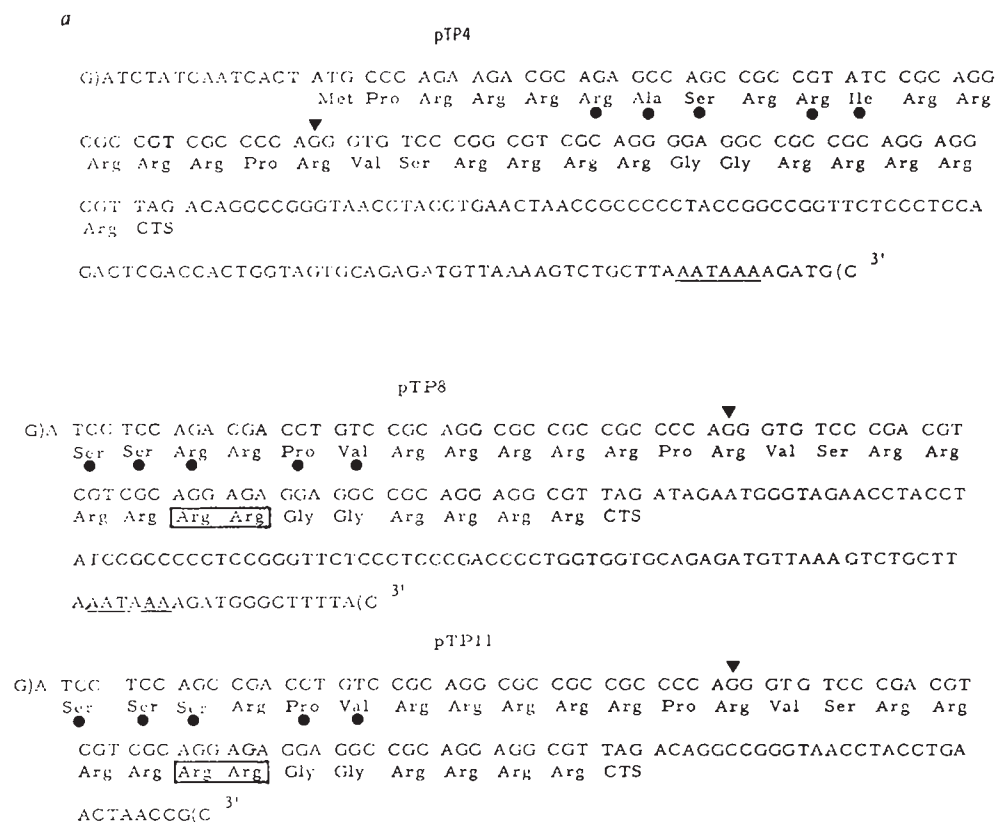


Fig. 3 Complete nucleotide sequences of the three protamine cDNA fragments carried in pTP4, pTP8 and pTP11, showing the encoded amino acid sequences. The cDNA fragments are flanked by dG·dC homopolymer linkers which are represented here by G).....(C). CTS marks the functional chain termination signal. Amino acid differences between the cDNA encoded sequences are indicated by ●. Residues in pTP8 and pTP11 corresponding to the deletion in pTP4 are boxed. **b**, Rainbow trout protamine sequences as determined by Ando and Watanabe³. ▼ Indicates the positions at which there is a common difference between the cDNA encoded and the published sequences.

b

1a Pro Arg₄ Ser Ser Ser Arg Pro Val Arg₅ Pro Arg₂ Val Ser Arg₆ Gly Gly Arg₄
 1b Pro Arg₆ Ser Ser Ser Arg Pro Ile Arg₄ Pro Arg₂ Val Ser Arg₅ Gly Gly Arg₄
 2 Pro Arg₄ Ser Ser Ser Arg Pro Val Arg₄ Ala Arg₂ Val Ser Arg₆ Gly Gly Arg₄

pTP4 5' AGAGCCAGCCGCGGTATCCGCAGGCCGCGTGGCCCCAGGGTGTCCCGCGGTCCG C
 pTP8 TCCTCCAGACGACCTGTCCGCAGGCCGCGCGCCCCAGGGTGTCCCGACGTCTCGC
 pTP11 TCCTCCAGACGACCTGTCCGCAGGCCGCGCGCCCCAGGGTGTCCCGACGTCTCGC

pTP4 AGGGGAGGCGCGCCGAGGAGGCGTTAG/ ACAGGCCGGGTA ACCTACCTGAACCT
 pTP8 AGGAGAGGAGGCCGAGGAGGCGTTAG/ ATAGAAATGGGTAGAACCTACCTGACCT
 pTP11 AGGGGAGGCGCGCCGAGGAGGCGTTAG/ ACAGGCCGGGTA ACCTACCTGAACCT

pTP4 AACCGCCCCCTACCGCCCGGTTCTCCCTCCAGACTCGACCACTGGTGGTGACAGGT
 pTP8 ATCCGCCCCCT CCGGGTTCTCCCTCC CGACCCCTGGTAGTGTAGAGAT
 pTP11 AACCG(C^{3'})

pTP4 GTTAAAGTCTGCTTAAATAAAAGATG(C^{3'})
 pTP8 GTTA AAGTCTGCTTAAATAAAAGATGGGCTTTTA(C^{3'})

Fig. 4 Composition of the shared nucleotide sequences of the protamine cDNA fragments carried in pTP4, pTP8 and pTP11. Coding sequences are separated from non-coding by /. Base transitions are represented by ●. Gaps in the sequence correspond to putative deletions required to give maximum alignment of sequences. Note that 29 nucleotides have been omitted from the 5'-end of pTP4.

possible arginine codons. The sequence data present a completely different picture. The arginine codons CGC and AGG are significantly over-represented, as is the serine codon TCC. CpG frequency in the coding regions of all three clones is that statistically expected from their G+C content and the dinucleotide appears exclusively in the arginine codon series CGX, which accounts for well over half the arginine codons in these sequences. However, in the 3'-non-coding regions, CpG frequency is depressed by about 50% compared to that expected. Methylation of DNA in eukaryotes seems to occur predominantly as 5-methyl CpG²⁷, and it has been suggested¹² that this may be the reason the dinucleotide is under-represented, for in bacteria at least, 5-methyl C residues are mutational hot spots probably due to their spontaneous deamination to thymine²⁸. The base transitions in these protamine cDNA fragments, however, fail to implicate CpG in sequence diversion, as there is only one instance of a C to T transition with G as its 3'-neighbour. C to T transitions in the first base of the arginine coding series CGX would generate the codons for cysteine and tryptophan or the chain terminator TGA. The presence of Cys and Trp in protamines could interfere with their function, thus selecting against C-T transitions in the DNA coding regions and it is significant that these amino acids are absent from both trout and the closely related herring protamines (clupeines)^{20,21}.

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Infectivity in mouse fibroblasts of polyoma DNA integrated into plasmid pBR322 or lambda phage DNA

THERE has been much speculation on the possible hazards of hybrid DNA technology¹. An important question in this context is whether a viral DNA, covalently linked to a vector, can be transferred from within a bacterium into an animal cell, either *in vitro* or *in vivo*, and initiate viral infection. As a first step, it is necessary to determine whether a hybrid containing eukaryotic viral DNA can, on penetration into a permissive cell, give rise to virus formation, as is the case for hybrids containing a prokaryotic viral sequence². As part of a risk-testing programme sponsored by EMBO, this question was investigated with mouse polyoma virus DNA. Polyoma virus will grow readily in mice; infection can be initiated by a single infectious particle as well as by naked viral DNA, and can be easily detected by monitoring the production of anti-viral antibodies. Virus production, and not tumour formation, was chosen as a criterion for biological activity because although polyoma virus will transform cells *in vitro*, tumours are produced *in vivo* only when large quantities of virus are inoculated into immunologically immature or immunosuppressed animals. We describe here the construction and characterisation of various plasmid pBR322 and phage λ derivatives containing polyoma DNA and their infectivity towards mouse fibroblast cells. The only recombinant molecules so far obtained which were infective as intact molecules were plasmids with dimeric polyoma DNA inserts.

Hybrid plasmid molecules were made with DNA from a readily identifiable mutant of polyoma³ (which lacked an *Hha* restriction site) by digestion with restriction enzymes *Eco*RI or *Bam*HI, each of which cleaves at a single site, and ligation to appropriately cleaved pBR322. The molar ratios of viral to plasmid DNA used were between 2:1 and 1:1. *Escherichia coli* HB101 was transformed with the recombinant DNA, and colonies containing polyoma DNA were identified by *in situ* hybridisation⁴ to ³²P-labelled polyoma RNA. Of 28 hybrid plasmids made by joining the constituent DNAs by their *Bam*HI sites, two had electrophoretic mobilities in agarose gels (0.5 and 0.52 relative to polyoma form I DNA) indicative of the presence of polyoma dimers. The remainder had higher relative mobilities (0.68-0.7) and were shown by analysis of restriction enzyme digests to contain one unit each of pBR322 DNA and polyoma, with both possible orientations being represented. The same type of analysis showed that the hybrid with a relative mobility of 0.5 comprised one molecule of pBR322 and two of polyoma DNA linked head-to-tail, whereas the hybrid with a relative mobility of 0.52 consisted of one molecule of pBR322 linked to a head-to-tail dimer of polyoma DNA with a deletion of about 800 base pairs. None of 48 hybrids of pBR322 and polyoma DNA joined through their *Eco*RI sites contained more than one molecule of polyoma DNA, but two contained two pBR322 molecules and both orientations of polyoma DNA with respect to pBR322 were found. Figures 1 and 2 show the analyses of

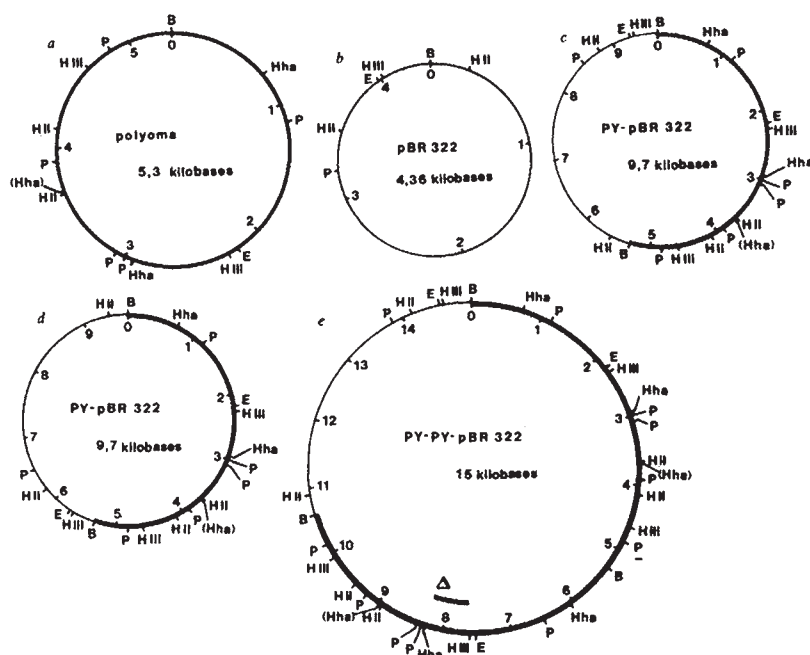


Fig. 1 Restriction maps of polyoma DNA, pBR322 DNA and some polyoma-pBR322 recombinant DNAs. *a*, Polyoma DNA (5,307 base pairs; from ref. 17). The DNA used here lacked the *HhaI* site (in parentheses) at position 26.4 from the *EcoRI* site. *b*, pBR322 DNA (4,361 base pairs; G. Sutcliffe and W. Gilbert, personal communication). *c*, Monomeric polyoma-pBR322 hybrid, joined at the *BamHI* sites so that the reading direction of the 'early' genes of polyoma and the *Tet* gene of pBR322 coincide (defined as head-to-tail). *EcoRI* cleavage should yield fragments of 7,075 and 2,575 base pairs. *d*, As *c*, except the reading directions are opposed (defined as head-to-head). *EcoRI* cleavage should yield fragments of 6,175 and 3,475 base pairs. *e*, Dimeric polyoma-pBR322 hybrid, joined at *BamHI* sites so that all units are head-to-tail, as defined above. Cleavage by *EcoRI* should yield fragments of 7,075, 5,300 and 2,575 base pairs. The other three possible combinations of orientations would yield the following *EcoRI* fragments: pBR322-Py (head-to-head)-Py (tail-to-head), 6,175, 5,300 and 3,475 base pairs; pBR322-Py (head-to-tail)-Py (head-to-head), 6,200, 6,175 and 2,575 base pairs; pBR322-Py (head-to-head)-Py (tail-to-tail), 7,075, 4,400 and 3,475 base pairs. B, *BamHI*; PstI, *PstI*; E, *EcoRI*; HII, *HindII*; HhI, *HhaI*; Hh, *HhaI*. Δ , site of deletion in hybrid D₅.

some of these plasmids and their structures.

Polyoma DNA was also inserted into DNA from several bacteriophage λ derivatives, some of which could lysogenise their host, some which had temperature-sensitive repressors, and some which carried functional genes for phage-mediated recombination (*red*). Linear polyoma DNA, obtained by digestion with *EcoRI*, was ligated to the restricted vector DNA, and viable phage DNA molecules were recovered by *in vitro* packaging⁵. Recombinant phage were distinguished from parental ones on the basis of plaque morphology, or their inability to permit synthesis of β -galactosidase (as demonstrated by their failure to generate blue plaques on an appropriate plate) in their infected host⁶, or by plaque hybridisation with ³²P-labelled polyoma RNA⁷. Plaques of recombinants were picked and taken through at least two cycles of purification from individual plaques before preparation of stocks by lytic infection. DNA from purified phage was analysed by digestion with restriction enzymes and electrophoresis in agarose gels (Fig. 3), and by electron microscopy of heteroduplexes formed

with DNA from their parent vector, or another recombinant. All but one of the recombinants analysed contained a unit length insert of polyoma DNA and both orientations with respect to each vector were represented; the exception, λ -Py 48, contained less than a unit length of polyoma DNA, the deletion being located at one of the junctions between polyoma and vector. Some of the recombinants obtained are described in Fig. 4. Various approaches failed to yield any stable recombinants containing more than one molecule of polyoma DNA, although biological screening on the basis of restriction coefficient to *EcoRI* (ref. 8) and ability to plate on *pel*⁻ (ref. 9) host strains indicated that several of the phage initially recovered contained dimers of polyoma DNA which were lost on further growth and purification. The reasons for this instability are unknown, but with the same vectors stable recombinants containing two molecules of pBR322 linked head-to-tail were obtained.

Recombinants were also made in λ derivatives with polyoma DNA cleaved at sites other than that for *EcoRI*. Partial digests of polyoma DNA with *HindIII* (for which it has two targets) were ligated with restricted DNA from two λ vectors^{6,22} (Fig. 4)

Table 1 Infectivity of polyoma-pBR322 and polyoma- λ hybrids

DNA preparation		Specific infectivity			
Prep. no.	Units of polyoma DNA per hybrid	Uncleaved DNA		Restricted DNA	
		PFU per μ g total DNA	PFU per molecule	PFU per μ g full-length polyoma DNA ($\times 10^{-4}$)	PFU per complete linear polyoma molecule ($\times 10^7$)*
<i>a</i> , Polyoma-pBR322 hybrids					
A ₂	1	<1	$<10^{-11}$	3.2	1.9
D ₆	1	<1	$<10^{-11}$	2.8	1.6
E ₈	1	<1	$<10^{-11}$	2.6	1.5
G ₃	1	<1	$<10^{-11}$	3.7	2.2
D ₃	2	7.5×10^4	1.2×10^{-6}	2.3	1.4
D ₅	1.9	8.7×10^3	1.4×10^{-7}	2.2	1.3
Py	—	2.2×10^6	1.3×10^{-5}	7.9	4.6
<i>b</i> , Polyoma- λ hybrids					
λ Py12	1	<1	$<10^{-10}$	1.6	0.94
λ Py35	1	<1	$<10^{-10}$	2.6	1.5
λ Py37	1	<1	$<10^{-10}$	1.8	1.1
λ Py48	<1	<1	$<10^{-10}$	$<10^{-4}$	$<6 \times 10^{-5}$
λ Py57	1	<1	$<10^{-10}$	2.3	1.4
λ PyPy	—	9×10^5	5.2×10^{-6}	6.4	3.8

The DNA was cleaved to completion with *BamHI* endonuclease (*a*) or *EcoRI* (*b*) and the enzyme was inactivated by heating at 60 °C for 15 min. Appropriate dilutions of unrestricted and restricted DNA, respectively, were assayed on mouse embryo cells for plaque formation using the DEAE method¹⁰. Py, polyoma DNA.

* The molecular weights (MW) used for the calculations are polyoma DNA, 3.51 $\times 10^6$; pBR322, 2.89 $\times 10^6$; λ DNA, 32 $\times 10^6$.

and again, two derivatives were used, one able to lysogenise its host and the other not. None of the recombinants isolated contained more than one molecule of polyoma DNA, but all contained the full monomer and with each vector three of the four possible derivatives were isolated (Fig. 4).

The infectivity of the polyoma-pBR322 hybrid DNAs before and after cleavage with the restriction enzyme used to generate the joined fragments was compared with that of polyoma virus DNA, using the DEAE method for transfection of mouse embryo cells¹⁰. Table 1 shows the specific infectivities of some of the polyoma-pBR322 hybrids joined through the *Bam*HI site. Hybrids containing one unit length of polyoma virus DNA gave less than 1 plaque-forming unit (PFU) per μg of hybrid DNA (less than 1 PFU per 10^{11} DNA molecules), but cleavage of these hybrid DNAs with *Bam*HI resulted in a specific infectivity (in terms of unit lengths of polyoma DNA) similar to that of the viral DNA cleaved with *Bam*HI. Similar results were obtained with five of six hybrids that contained monomers of polyoma virus DNA joined to pBR322 through the *Eco*RI cleavage site

(results not shown). In no case was the uncleaved DNA infectious, but five of the hybrid DNAs were infectious after cleavage with *Eco*RI, which liberated a unit length linear viral genome. The reason for the lack of infectivity of the remaining hybrid after restriction remains uncertain; a non-infectious molecule may have been cloned, or infectivity of the polyoma DNA moiety may have been lost on passage of the hybrid in bacteria.

In contrast to the intact hybrids containing one unit of polyoma DNA, those containing 2 or 1.9 tandem head-to-tail genomes of polyoma virus DNA were infectious. The hybrid containing a dimer of polyoma virus DNA was 10% as infectious as intact form I viral DNA (in terms of PFUs per polyoma DNA molecule), whereas the hybrid containing 1.9 viral genomes was about 1% as infectious as viral DNA. The specific infectivity after cleavage with *Bam*HI of the two hybrids containing more than one unit of polyoma virus DNA was similar to that of similarly treated viral DNA in terms of PFUs per unit length of polyoma DNA molecules.

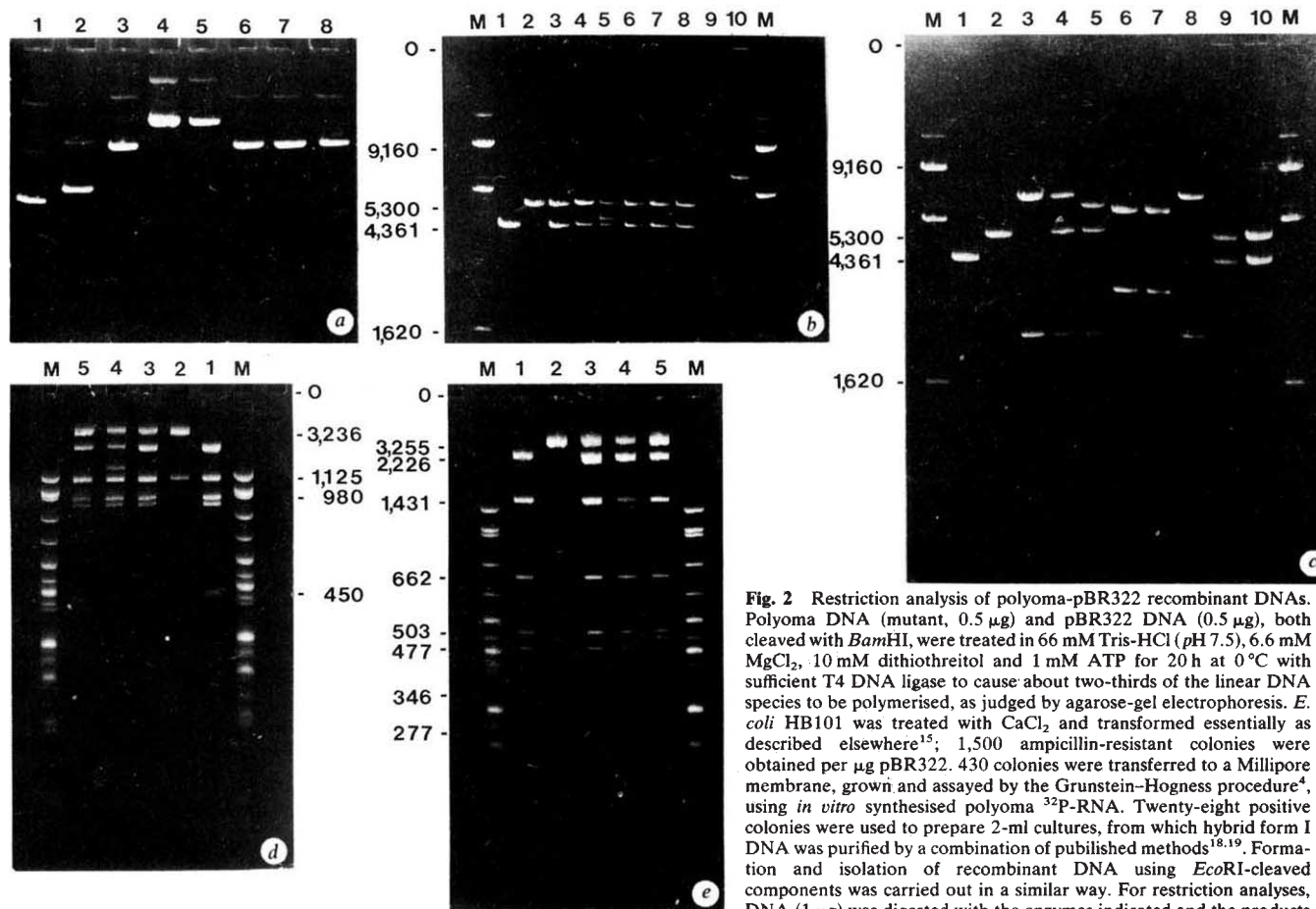


Fig. 2 Restriction analysis of polyoma-pBR322 recombinant DNAs. Polyoma DNA (mutant, 0.5 μg) and pBR322 DNA (0.5 μg), both cleaved with *Bam*HI, were treated in 66 mM Tris-HCl (pH 7.5), 6.6 mM MgCl_2 , 10 mM dithiothreitol and 1 mM ATP for 20 h at 0°C with sufficient T4 DNA ligase to cause about two-thirds of the linear DNA species to be polymerised, as judged by agarose-gel electrophoresis. *E. coli* HB101 was treated with CaCl_2 and transformed essentially as described elsewhere¹⁵; 1,500 ampicillin-resistant colonies were obtained per μg pBR322. 430 colonies were transferred to a Millipore membrane, grown and assayed by the Grunstein-Hogness procedure⁴, using *in vitro* synthesised polyoma ³²P-RNA. Twenty-eight positive colonies were used to prepare 2-ml cultures, from which hybrid form I DNA was purified by a combination of published methods^{18,19}. Formation and isolation of recombinant DNA using *Eco*RI-cleaved components was carried out in a similar way. For restriction analyses, DNA (1 μg) was digested with the enzymes indicated and the products analysed by electrophoresis on agarose gels in the presence of ethidium bromide²⁰. a, Native DNA samples: (1) pBR322 (the major band is form I DNA, the minor bands are probably due to form II and to spontaneous pBR322 dimers, forms I and II); (2) Polyoma DNA; (3-8) hybrids A_2 , D_3 , D_5 , D_6 , E_8 , G_3 ; b, *Bam*HI cleavage. (1) pBR322; (2) polyoma; (3-8) hybrids linked through *Bam*HI sites: A_2 , D_3 , D_5 , D_6 , E_8 , G_3 ; (9, 10) hybrids linked through *Eco*RI sites: A_{91} , B_{46} ; M, marker: ϕ 29 DNA incompletely digested with *Eco*RI. c, *Eco*RI cleavage. Lanes numbered as in b. d, Double digestion with *Pst*I and *Bam*HI. (1) Polyoma DNA; (2) pBR322; (3, 4) hybrids with more than one unit polyoma linked through *Bam*HI sites: D_3 , D_5 ; (5) monomeric hybrid linked through *Eco*RI site: E_8 ; M, marker: pCRI DNA digested with *Bsp*I. e, Triple digestion with *Hind*II, *Hind*III and *Bam*HI. Lanes numbered as in d. One of the larger ('dimeric') hybrids (D_3) gave two fragments on digestion with *Bam*HI: the longer one, which had the mobility of linear polyoma DNA, was present in about twice the molar yield (as estimated visually) of the shorter one, which had the mobility of linear pBR322. *Eco*RI cleavage of hybrid D_3 gave three products. The largest (7,100 base pairs) and the smallest (2,600 base pairs) of these had the same mobility as the *Eco*RI fragments of hybrids A_2 and G_3 , respectively, whereas the third fragment (5,300 base pairs) co-migrated with linear polyoma DNA. The hybrid D_3 therefore consists of one molecule of pBR322 and two of polyoma DNA linked head-to-tail, in the orientation shown in Fig. 1e. The other large (dimeric) hybrid D_5 gave three fragments on cleavage with *Bam*HI, one with the mobility of pBR322, one with the mobility of polyoma DNA, and one with a mobility corresponding to a chain length of about 4,700 base pairs. Thus, one of the polyoma DNA units has a deletion of about 600 base pairs. Digestion of D_5 with *Eco*RI yielded the same two smaller fragments (5,300 and 2,600 base pairs) as did the dimeric polyoma plasmid D_3 ; however, the largest (7,100 base pair) fragment was replaced by a shorter one of about 6,400 base pairs (c). As shown in Fig. 1e yields fragments of 5,300 and 2,600 base pairs; therefore, D_5 probably consists of one molecule of pBR322 linked to a head-to-tail polyoma dimer with a deletion of about 800 base pairs in the region yielding the large *Eco*RI fragment. The site of the deletion was located more precisely by analysing the products obtained after simultaneous digestion with *Bam*HI and *Pst*I on the one hand and with *Bam*HI, *Hind*II and *Hind*III on the other. The resulting fragments were compared with those from a similar digestion of polyoma DNA, pBR322 and the dimeric polyoma plasmid D_3 . As shown in d, hybrid D_5 doubly digested with *Bam*HI and *Pst*I, lacked a 1,850-base pair fragment specific to polyoma which was replaced by a shorter fragment of about 1,300 base pairs; after digestion with *Bam*HI, *Hind*II and *Hind*III one (of two) polyoma-specific fragments of 1,450 base pairs was replaced by one of ~700 base pairs. No pBR322-specific bands were missing. The deletion is thus located at the position indicated in Fig. 1e. The orientation of the A_2 and G_3 hybrids is indicated in Fig. 1c and the D_6 and E_8 hybrids in Fig. 1d.

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Polyoma virus was isolated from several cell cultures that had been infected with the restricted plasmid containing monomeric polyoma DNA or with the unrestricted plasmid containing dimeric polyoma DNA; in all cases, the progeny had only the two *HhaI* sites characteristic of the mutant DNA used to produce the recombinant DNAs. To eliminate the possibility that free polyoma DNA (linear or circular) was present in the hybrid DNA preparations before transfection, 1- μ g samples of the unrestricted hybrids containing dimers of polyoma DNA were subjected to agarose-gel electrophoresis. The DNA was denatured and transferred from the gel to cellulose nitrate membranes¹¹ and polyoma-specific sequences were located by hybridisation with ³²P-labelled polyoma virus DNA followed by autoradiography. No radioactive band was detected in the positions of forms I or II polyoma DNA; 0.1 ng of polyoma DNA could be detected in parallel lanes. This experiment shows that the observed infectivity was due to the polyoma-pBR322 hybrid molecules and not to free viral genomes in the preparations, as a possible contamination could not exceed about 0.01%.

The results for the infectivity of the polyoma- λ hybrids before and after restriction were essentially the same as those for the monomer polyoma-pBR322 hybrids. Less than 1 PFU per μ g of hybrid DNA (<1 PFU per 10^{10} DNA molecules) could be detected after infection of mouse cells with any of the uncleaved polyoma- λ hybrids that contained a single copy of the polyoma virus genome, but cleavage with the appropriate restriction enzyme led to an infectivity similar to that of a molar equivalent of restricted polyoma DNA (Table 1). Of the polyoma- λ hybrids constructed through *EcoRI* sites, seven of the eight different types of DNA molecule were infectious after digestion with *EcoRI*, but DNA from one hybrid (λ -Py 48), which was shown to have a deletion at one of the sites of attachment, was not infectious after such treatment. Similarly, the hybrids constructed through the *HindIII* sites, one of which is in the early and one in the late region, were not infectious as intact DNA molecules.

These experiments show that it is possible to prepare and maintain in *E. coli* strain HB101 plasmids containing two units

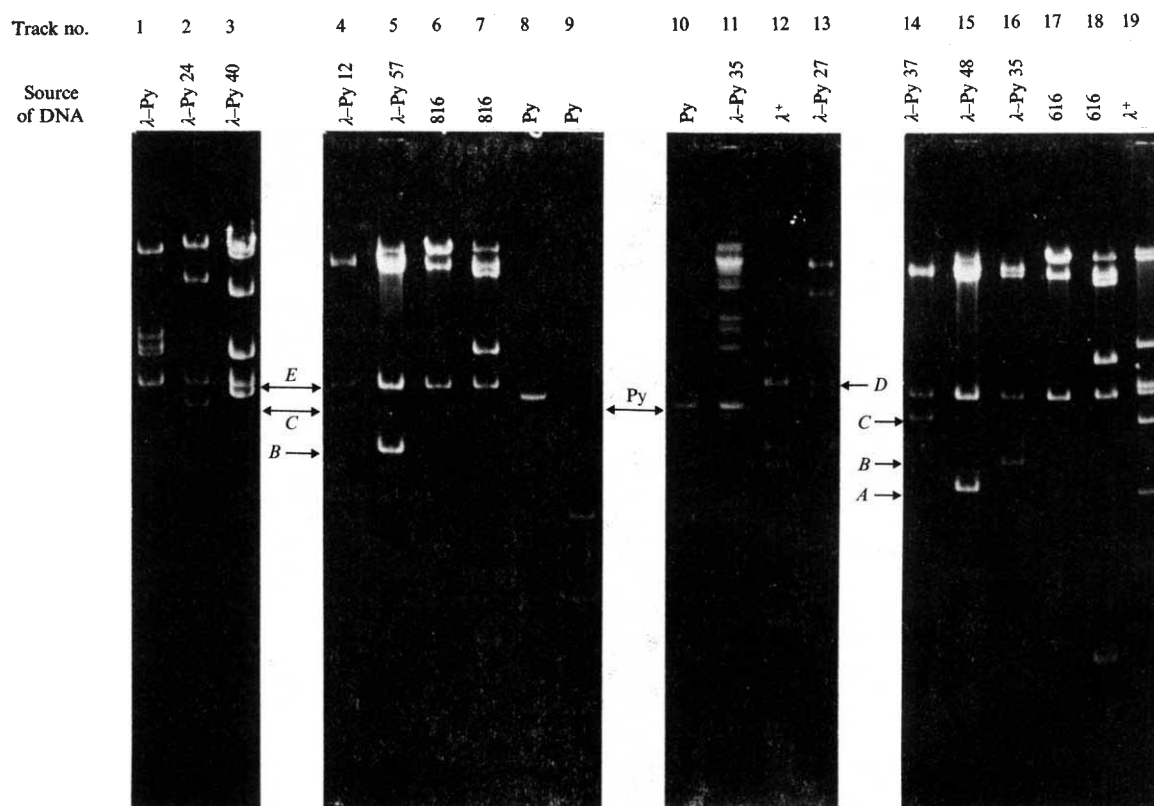
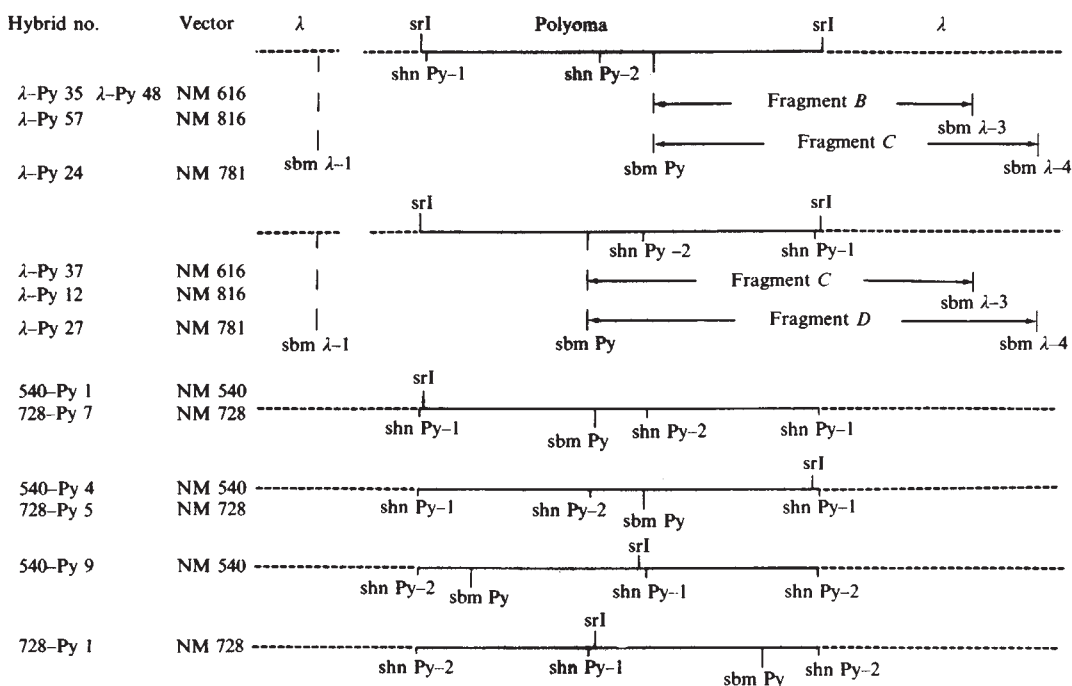


Fig. 3 Electrophoresis of DNA from λ -Py hybrids in 1% agarose gels. The samples were digested with the following enzymes: tracks 1-7 and 13-17, *Bam*HI; tracks 8, 10, 11, 19, *Eco*RI; tracks 9, 18, *Bam*HI and *Eco*RI; track 12, *Hind*III and *Eco*RI. The samples in tracks 1, 9, 12 and 19 serve as reference for the size of fragments; the samples in tracks 11 and 12 show over-digestion with *Eco*RI, but nevertheless clearly show the release of unit length Py DNA from λ -Py 35 on digestion with *Eco*RI and provide the necessary reference points for known fragments of λ^+ DNA in the digest with *Hind*III and *Eco*RI (refs 20 and 22). The hybrid phage were made as follows. The vector DNA (usually 1 μ g) was digested with the appropriate restriction enzyme and incubated with Py DNA (about 0.5 μ g digested with the same restriction enzyme) and T4 DNA ligase (0.1 units) in 66 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 1 mM EDTA, 40 mM NaCl, 10 mM 2-mercaptoethanol and 0.1 mM ATP (0.1 ml) at 10 °C for 3-6 h and then 0 °C for at least 24 h and sometimes several days. In some experiments linear dimers of Py DNA were isolated by agarose-gel electrophoresis of samples of Py DNA (10 μ g) that had been digested with *Eco*RI, heated to inactivate the enzyme and then incubated with T4 ligase. The section of gel containing the linear dimers was dissolved in saturated potassium iodide solution²¹ and the DNA adsorbed on hydroxyapatite from which it was recovered by elution with 0.4 M potassium phosphate, pH 7, and dialysed against 0.3 M NaCl and then 10 mM Tris-HCl, pH 8, and 1 mM EDTA. Linear Py DNA cleaved at one of the two *Hind*III targets was prepared by partial digestion in conditions previously shown to give no detectable fragments of Py DNA. Viable phage DNA molecules were recovered by transfection of competent cultures¹⁵ of *E. coli* $r_k^- m_k^- recA$ prepared as described by Lederberg and Cohen¹⁶ or, more frequently, by *in vitro* packaging using cells that had been UV-irradiated⁵. Plaques screened as indicated in Fig. 4 were picked into phage buffer containing CHCl₃ and re-plated. After two such cycles, plate lysates were prepared and used to infect small-scale liquid cultures. From these lysates 1-l cultures were prepared by infection of cells at a m.o.i. of 1 followed by growth for 3 h with vigorous aeration. Lysis was completed by addition of CHCl₃ and phage recovered from the clarified lysates by precipitation with polyethylene glycol (Carbowax 6000, to 10% w/v, NaCl to 0.5 M) overnight at 4 °C. The precipitates were recovered by centrifugation, extracted with phage buffer and after clarification by centrifugation at 10,000 r.p.m. the phage were isolated by centrifugation on CsCl step gradients followed by equilibrium centrifugation in 41.5% w/w CsCl (ref. 14). Phage bands were collected and dialysed against 10 mM Tris-HCl, pH 8 and DNA prepared by phenol extraction followed by dialysis. Samples were digested with restriction enzymes as indicated above and analysed by electrophoresis in agarose gels (1% in 40 mM Tris-acetate, pH 8.2) which were photographed under UV light after staining with ethidium bromide (0.5 μ g ml⁻¹). In some cases the gels were soaked in NaOH solutions to denature DNA fragments for transfer to cellulose nitrate membranes¹¹ and hybridisation with ³²P-labelled Py RNA. The fragments A, B, C and D all hybridised with the Py probe. The MW of these fragments deduced from their electrophoretic mobilities are: A, 2.5×10^6 ; B, 2.75×10^6 ; C, 3.25×10^6 ; D, 3.9×10^6 . Fragment E is characteristic of *Bam*HI digests of DNA from λ derivatives and is the left terminal fragment of the molecule. All operations from transfection or packaging of ligase reaction products to isolation and restriction digestion of the hybrid phage DNA were carried out in a Category III laboratory and all cultures and lysates were inoculated and handled in glove boxes, rather than laminar flow cabinets. Digested DNA was analysed in Category I laboratories.

Fig. 4 Orientation of polyoma DNA within λ in the λ -Py hybrids. Only part of the λ genome is included in the diagrams showing the orientation of the polyoma DNA within λ . The vectors are described elsewhere^{6,22,23}; NM 816 is a cI^{ts} derivative of NM616. Targets for *EcoRI* are denoted by *srI*, and targets for *BamHI* and *HindIII* by *sbm* and *shn*, respectively. The orientations were based initially on the size of the fragments (determined from their electrophoretic mobility in 1% agarose gels) released by digestion with *BamHI*. In the case of the hybrids made through the *EcoRI* sites, the important fragments were A, B, C and D (Fig. 3), but fragment B (2.75×10^6 daltons) and fragment D (3.9×10^6 daltons) are the same size as fragments that would be released by the action of *BamHI* on head-to-head or tail-to-tail dimers of polyoma (2.8 or 3.9×10^6 daltons). Heteroduplexes made between all the hybrid DNAs and their parent vectors showed, however, that all but one of the hybrids contained an inserted DNA fragment equal in length to polyoma DNA. The exception was λ -Py 48, which had a slightly smaller fragment inserted. The fragment in the *BamHI* digest of λ -Py 40 (Fig. 3, track 3) with the same mobility as polyoma DNA was, in fact, not polyoma DNA, but a fragment containing both λ and polyoma sequences; the orientation of the polyoma DNA in this hybrid is not shown here. Heteroduplexes between pairs of hybrids confirmed that the polyoma inserts in λ -Py 35, λ -Py 48 and λ -Py 57 were in the same orientation, but in the opposite orientation from those in λ -Py 37 and λ -Py 12; similarly, the orientation of the polyoma in λ -Py 24 was shown to be opposite to that in λ -Py 27. The heteroduplexes between λ -Py 35 and λ -Py 48 showed a deletion of about 0.7% of the total DNA located at 59% from one end of the molecule. This, together with the MW of fragment A in a *BamHI* digest (2.5×10^6), showed the presence of a deletion of about 0.25×10^6 daltons (about 350 base pairs) in hybrid λ -Py 48 and digestion of the DNA with *EcoRI* showed that the deletion removed one of the *EcoRI* sites (*srI*A-2) at which the polyoma and vector DNAs had been joined. Further digests of the hybrid DNA preparations with *HindIII* and digests with both *EcoRI* and *BamHI* (results not shown) confirmed these conclusions and assignments of orientation. The orientations of the polyoma DNA in the λ -Py hybrids made through *HindIII* sites and the determination of the *HindIII* site in the polyoma DNA at which the insertion had occurred were confirmed by an extensive analysis (by gel electrophoresis) of the digestion products formed with *BamHI*, *HindIII* and *EcoRI*, acting singly and in combination (results not shown).



of polyoma DNA linked head-to-tail at the *BamHI* site. The failure to recover hybrids that contain dimeric polyoma DNA linked to plasmid DNA or to phage λ DNA through the *EcoRI* site is not understood although the changes in characteristics observed during the propagation of some of the polyoma- λ hybrids recovered by *in vitro* packaging suggest that phage genomes containing dimeric polyoma DNA were formed but were unstable. It seems that with both *red⁺* and *red⁻* phage in *rec⁺* or *recA* host cells the dimeric polyoma DNA is converted rapidly into monomer size by some recombinational events in the bacterial cell. None of the hybrid phage constructed through the *HindIII* site contained dimers of polyoma DNA. However, more recent results suggest that phage containing dimers of polyoma DNA can be made through the *BamHI* site although their stability is unknown.

The fact that cloned polyoma DNA, when excised at the site of joining, has an infectivity very similar to that of form III DNA derived from polyoma virions is evidence for the remarkable fidelity of the cloning procedure and the stability of a eukaryotic sequence replicated in a prokaryotic host. The finding that hybrid DNA containing only one unit length of polyoma DNA is not infectious to cells permissive for polyoma shows that intracellular excision of full-length, circular or linear polyoma DNA from the hybrid must be a very rare event. In contrast, excision of polyoma DNA from a hybrid containing a head-to-tail dimer is relatively efficient in mouse fibroblasts and probably results from legitimate recombination. A hybrid containing a head-to-tail dimer of polyoma DNA with an 800-base pair deletion in which, due to the location of the deletion (Fig. 1e), only 1.3 units of polyoma DNA are available for excision of a unit length viral DNA, had 10% of the infectivity of the plasmid containing the full dimer. This suggests that the frequency of

excision may be related to the target size for possible recombinational events. The production of SV40 virus from adenovirus-SV40 hybrids has also been observed to depend on the extent of duplicated SV40 sequences present²⁴. The observed infectivity of hybrid DNA containing dimers of polyoma DNA resembles the results obtained by Jaenisch and Levine¹², and Israel *et al.*¹³, who found that linear and circular oligomeric SV40 DNA was infectious to permissive cells and gave rise to SV40 particles containing monomeric DNA.

Experiments designed to test the transfer of recombinant DNA from *E. coli* to an animal cell or to an animal can only be useful if the recombinant DNA itself is potentially infectious. The demonstration that hybrid DNA containing head-to-tail dimeric polyoma DNA is highly infectious to mouse fibroblasts whereas that containing a monomeric DNA insert is not, justifies an extension of the experiment to *in vivo* studies. In these studies bacteria containing such hybrid plasmids and phage will be administered to cultured mouse cells and to mice which will then be scored for polyoma infection.

After completion of this work two reports were published on the cloning of polyoma virus DNA in the pBR322 plasmid and bacteriophage λ in the enfeebled *E. coli* host χ 1776 (refs 25, 26). In one case a dimer of polyoma DNA was shown to have been cloned in λ but in an unstable fashion. Consistent with the results presented here that recombinants containing duplicated polyoma virus sequences are biologically active *in vitro*, these workers found that only recombinant DNA preparations containing dimer polyoma sequences were biologically active when injected into animals.

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Cytoplasmic synthesis of plastid polypeptides may be controlled by plastid-synthesised RNA

It is generally agreed that some plastid polypeptides are coded by plastid DNA and the remainder by nuclear DNA, and that some plastid polypeptides are synthesised on plastid ribosomes and the remainder on cytoplasmic ribosomes^{1,2}. Studies on two mutant lines of barley (*Hordeum vulgare* L. 'albostrians' (M4205) and 'Saskatoon') have helped the understanding of the integration of the plastid and nucleocytoplasmic systems. In both lines a nuclear mutation is expressed as an irreversible mutation of the plastid DNA yielding pigment-deficient plastids whose segregation leads to white-striped leaves and later to pure white leaves and shoots^{3,4}. The white leaves do not contain

plastid ribosomes and no protein synthesis can be detected in the plastids, although cytoplasmic ribosomes are present in normal amounts^{5,6}. Immunoelectrophoresis of extracts of white leaves has failed to detect either the plastid-synthesised large subunit or the cytoplasmically synthesised small subunit of ribulose-bisphosphate carboxylase/oxygenase, or coupling factor CF₁, which has three plastid-synthesised and two cytoplasmically synthesised subunits, or the cytoplasmically synthesised ferredoxin-NADP⁺ reductase^{6,7}. We report here that two cytoplasmically synthesised plastid enzymes⁸, phosphoribulokinase (EC 2.7.1.19) and D-glyceraldehyde-3-phosphate: NADP⁺ oxidoreductase (phosphorylating) (EC 1.2.1.13), occur in considerably reduced amounts in white leaves and deduce how the plastid may control the cytoplasmic synthesis of plastid polypeptides.

Enzyme activities were determined in the clear supernatants of homogenates made from barley leaves and were carried out for each enzyme both before and after *in vitro* activation, as described in Table 1 legend. Normally pigmented leaves of M4205 gave results for the two enzymes similar to those obtained previously for several other species, in that *in vitro* activation was substantial in extracts from etiolated leaves but proportionately smaller in extracts from illuminated green leaves because illumination of green leaves causes activation *in vivo*⁹. Table 1 shows that plastid pigment-deficient leaves contained measurable amounts of both enzymes and that the activities increased in response to *in vitro* activation. Thus the enzymes were present in both dark-grown leaves and in leaves subjected to 48 h of illumination, their activities falling within a range of 0.5-3.6% of those of the normally pigmented leaves of M4205. Absorption spectra and their second derivatives of intact white leaves have shown the presence of small amounts of carotenoids and chlorophyll at less than 1% of the plastid pigment content of normally pigmented leaves of M4205 (T.B. and A. Meister, unpublished). Parallel experiments with 'Saskatoon' barley yields results similar to those shown in Table 1 for M4205. Note that the *Euglena gracilis* mutant W₃BUL, which lacks plastid DNA, also contains very low amounts of cytoplasmically synthesised plastid polypeptides¹⁰.

Plastid pigment-deficient leaves of both mutant barley strains seem to show slightly 'leaky' inhibition of the cytoplasmic formation of phosphoribulokinase and glyceraldehyde-phosphate dehydrogenase (NADP⁺). This contrasts with data for genetically normal seedlings grown at high non-permissive temperatures (28-34 °C) where the newly formed parts of the leaves are free from plastid ribosomes and deficient in plastid pigments¹¹. The non-permissive temperature may function by preventing the assembly of plastid ribosomes¹². Pigment-

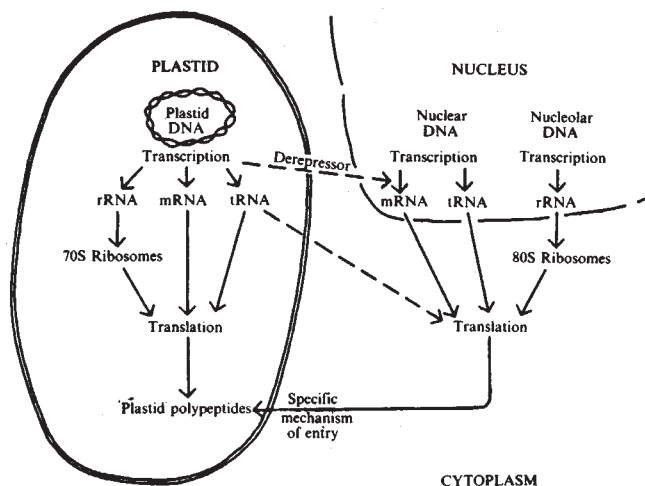


Fig. 1 Two alternative possibilities for the control of the cytoplasmic synthesis of plastid polypeptides by RNA transcribed from the plastid genome (effects expressed by broken lines).

Table 1 Comparison of the phosphoribulokinase and glyceraldehyde-phosphate dehydrogenase (NADP⁺) activities of leaves of the barley mutant 'albastrians' (M4205) which either possess or are deficient in plastid pigments

	Enzyme activity (units per g fresh weight)			
	Phosphoribulokinase Without <i>in vitro</i> activation	Phosphoribulokinase With <i>in vitro</i> activation	Glyceraldehyde-phosphate dehydrogenase (NADP ⁺) Without <i>in vitro</i> activation	Glyceraldehyde-phosphate dehydrogenase (NADP ⁺) With <i>in vitro</i> activation
7 d dark growth				
Plastid pigments normal	9.7	12.3	3.2	4.3
Plastid pigments deficient	0.15	0.37	0.09	0.15
7 d dark growth plus 2 d illumination				
Plastid pigments normal	49	51	14.1	16.6
Plastid pigments deficient	1.2	1.8	0.07	0.22

Two barley seedling first leaves were weighed and then homogenised in 5 ml of ice-cold extraction medium (100 mM Tris-HCl, pH 7.8 and 2 mM dithiothreitol) for 3 min in a Tenbroek homogeniser and the enzyme activities were determined in the supernatant obtained after 1 min centrifugation at 5,000g. Both enzymes were assayed spectrophotometrically from NAD(P)H consumption measured at 344 nm and 30 °C as previously described⁹. Phosphoribulokinase was activated by 12 min preincubation of 0.1 ml of extract with 50 µmol Tris-HCl, pH 7.8, 2 µmol dithiothreitol, 10 µmol MgCl₂, 2 µmol ATP and 40 µmol KCl at 30 °C in a total volume of 0.38 ml. Glyceraldehyde-phosphate dehydrogenase (NADP⁺) was activated by 5 min preincubation of 0.1 ml extract with 50 µmol Tris-HCl, pH 7.8, 10 µmol MgCl₂, 4.8 µmol EDTA, 3 µmol ATP and 5 µmol 3-phosphoglycerate at 30 °C in a total volume of 0.34 ml. Each value represents the mean from two separate leaf samples.

deficient sections of rye leaves grown at the non-permissive temperature (32 °C) contained normal amounts of the cytoplasmically synthesised photosynthetic carbon pathway enzymes, including phosphoribulokinase and NADP⁺-dependent glyceraldehyde-phosphate dehydrogenase, and furthermore these activities were found to accumulate within unpigmented plastids¹³. These pigment-deficient leaves also synthesised and accumulated the small subunit of ribulose-bisphosphate carboxylase/oxygenase in the absence of any synthesis of the large subunit¹⁴.

This work with seedlings grown at high temperatures established that the presence of functional chloroplasts is not necessary for the accumulation of cytoplasmically synthesised plastid stroma enzymes, and suggests that the failure of such enzymes to show appreciable accumulation in unpigmented leaves of the mutant barley was a result of the inhibition of their synthesis. As back-crossing experiments³ have shown that the nuclear genome of the mutant barley is stable and as the inhibition of the cytoplasmic synthesis of plastid polypeptides was not found until the plastid DNA became defective, it may be deduced that the inhibition of the cytoplasmic synthesis of plastid polypeptides resulted from the loss of a product or products, arising from plastid DNA, which controlled this process. As plastid pigment-deficient leaves of the mutants and of the high temperature-grown seedlings all lacked ribosomes and did not show polypeptide synthesis in the plastids, whereas the former lacked and the latter showed the cytoplasmic synthesis of plastid polypeptides, it seems evident that such a product could not have been a plastid-synthesised polypeptide or the metabolic product of the enzymatic activity of such a polypeptide. The pigment-deficient plastids of the mutants had undergone an irreversible change in their DNA, whereas the DNA of the plastids of the heat-treated seedlings is assumed to have been unaffected, as the effects of the heat treatment were reversed by return to a permissive temperature¹⁵.

The most direct explanation for the inhibition of the cytoplasmic synthesis of plastid polypeptides in the pigment-deficient barley mutants is the assumption that they are deficient in RNA transcribed from the plastid DNA. This RNA may function in derepressing the transcription of nuclear DNA coding for cytoplasmically synthesised plastid polypeptides. Alternatively it may function as tRNA necessary for the cytoplasmic translation of mRNA coding for plastid polypeptides in agreement with the recent report that 7–9 plastid DNA cistrons of *Euglena gracilis* code for tRNA molecules which function with cytoplasmic polyribosomes and which are distinct from the

tRNA which functions in polypeptide synthesis in the plastid¹⁶. These mechanisms, shown in Fig. 1, may be considered in relation to other proposals that organelle gene products may control the nucleocytoplasmic synthesis of organellar polypeptides for mitochondria¹⁷ and chloroplasts².

Our deductions are not compatible with the 'cytoplasmic control principle' as formulated by Ellis¹ for the regulation of organellar protein synthesis and which states that "cytoplasmic products control organellar protein synthesis, but that the converse does not occur". We do not dispute that cytoplasmic products may control organellar protein synthesis but cannot accept the second part of this statement. On the contrary, we consider that there are also cases in which plastid products control the amount of synthesis of plastid proteins on cytoplasmic ribosomes.

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matters arising

Short-term storage and wind power availability

ANDERSON *ET AL.*¹ present a further analysis of Ryle's suggestion on the role of alternative energy sources² that wind power could be used in conjunction with 150-h thermal storage to provide domestic space heating. Ryle contended that "as soon as such storage is introduced a number of alternative sources of energy can be compared on an equal basis with a nuclear system"; in other words, that wind power can not only replace nuclear power as an energy producer but, when used in conjunction with 150-h storage, can contribute just as reliably to meeting peak electricity demands. Our disagreement with Ryle is on the latter point.

In our previous paper³ and our comments on Diesendorf and Westcott⁴, we attempted to show that 150-h storage was inadequate for matching wind generator output to the heating load and as a result would have little, if any, effect in reducing the amount of firm generating capacity needed for meeting peak demands on the electricity supply system. We believe that the data presented by Anderson *et al.*¹ support this view.

Figure 1 of ref. 1 shows that over the 17-yr period considered, wind power in conjunction with 150-h storage would have failed to maintain room temperature to within 3 °C of the target temperature (20 °C) for 14% of the time, while Fig. 2 shows that during the period February–March 1975 the temperature would have fallen below 17 °C for 65% of the time. We contend that the occupants would resort to supplementary direct electrical heating during such periods with the result that the peak load on the supply system would be little different from what it would have been in the absence of wind generation. Consequently, unless storage of the type advocated by Ryle could be economically provided for periods much longer than 150 h, wind power would operate only as a fuel saver.

Anderson *et al.*¹ also suggest that the optimum ratio of rated to annual average wind speed for a wind turbine would be closer to a value of 1.5 than the 2.3 ratio used by ETSU in Energy Paper 21, which we also adopted for our estimates. We acknowledge that such a choice of ratio would reduce the time of zero output and hence the storage requirement. However,

an estimate we have made from an analysis of 16 yr of wind data from four of the sites of ref. 3 suggests that the reduction in energy output would be well above the estimate of 20% so far as the higher wind speed sites, including offshore locations, are concerned. For such sites a mean annual wind speed of 14 knots at the standard 10 m recording height would be more typical than the 12 knots on which calculations are based in ref. 1. We estimate that, in the former case, the energy loss would be closer to 40%, a reduction which would outweigh the savings on capital cost. We agree, however, that the economic optimum is likely to be somewhat lower than 2.3 even for very windy sites, the exact value being machine design dependent. Anderson *et al.* suggest that the energy deficit resulting from operation at a ratio of 1.5 can be regained by increasing the radius of the rotor. It would seem to us that having optimised the ratio of the rated to mean wind speed at a given site for a given design of machine, the size of rotor would be maximised within the constraints of engineering feasibility.

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ANDERSON *ET AL.* REPLY—Leicester *et al.* have for the most part accurately restated our quantitative results¹. With reference to the need for a supplementary supply, we would stress that the system we assumed was (intentionally) the most stringent in that, even with perfect correlation between available wind energy and heating demand, the house temperatures would only just be maintained at 20 °C. Nevertheless, the lowest temperature reached over the entire 17-year period was 10 °C, at a time when the outside temperature was ~0 °C. The system was, therefore, always able to supply at least 50% of the energy required (heating demand being assumed proportional to the difference between internal and external temperatures). Even if the energy shortfall on this isolated occasion

had to be met entirely by the conventional grid, the electrical heating demand would be reduced by ~50% over that necessary in the absence of wind power, which we would not describe as 'hardly different'.

The question of turbine rating has apparently not been fully appreciated by Leicester *et al.* Our analysis (which was based on calculations involving actual wind data and not just estimates) depends only on the statistics of the wind distribution at a particular site and is otherwise completely independent of the precise value of mean wind speed. The loss in total annual energy output for a change of turbine rating from 2.3 $\bar{V}$ to 1.5 $\bar{V}$ is relatively independent of the particular site and corresponding mean wind speed as can be seen from Fig. 7 of the ETSU report²; this figure, which shows the results for seven sites, including one of the higher wind speed sites referred to by Leicester *et al.*, indicates values of this loss in the range 15–25%, rather than the 40% estimated by Leicester *et al.* The specific value of 12 knots used in our Table 2 (ref. 1) was adopted simply to allow actual figures for power output and so on to be quoted; the conclusions would have been similar for any reasonable value of adopted mean wind speed.

The optimisation of costs for a particular design is a complex problem and the engineering constraints involve not only the size of the rotor but also, for example, the limitations of gearbox torque and tower strength. The same annual energy output can be obtained by the use of a larger but more lightly-loaded rotor operating over a lower range of wind speeds, allowing the use of a smaller gearbox and alternator and reducing the structural loadings of the tower. On the basis of the relative costs given by ETSU² this will usually lead to a more economical design.

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TO CONCLUDE this correspondence the authors have been invited to provide a

final summary of the main points of agreement and disagreement between them.

THERE are two principal points of disagreement: (1) the extent to which wind power (with 150-h storage) can provide a reliable heating supply; and (2) the variation of net power output with aerogenerator rating.

(1) Ryle¹ considered the question of domestic heating demand. He concluded that wind power with the addition of 150-h storage on the consumers' premises could meet this demand at all times and thus replace an equal amount of conventional or nuclear generating plant.

In an analysis covering a six-month period, Leicester *et al.* (ref. 2 and above) found periods of a week or more during which the 150-h storage, initially full, would have been completely discharged and therefore unable to meet any of the heating demand. They pointed out that at such times there would have been little reduction in the heating demand imposed on the electricity grid and, consequently, little saving in the amount of thermal plant needed to meet peak demands.

Anderson *et al.* later proposed³ a modified system with a store in which the heat output is a linear function of the energy remaining in the store. In this system as the heat output from the store fell short of that required the demand on the grid would progressively increase. They found that over a 17-year period this demand would never exceed about half the average heating load, with a corresponding reduction in the required installed thermal power station capacity. Thus, although a wind energy system in which there was no surplus capacity would be unable to supply the entire heating load, it could, nevertheless, make a significant power as well as energy contribution.

Leicester *et al.* agree that this second proposal is of interest, but further work will be required before the full implications of such a system can be assessed. (2) The disagreement on the second point is less easy to explain. Based on the analysis of wind data, the Cambridge group suggest that changing the rated speed of the aerogenerator from 2.3 to 1.5 times the mean site wind speed decreases the annual energy output by between 15 and 25% (in agreement with the data given in the ETSU paper) whereas the CEGB group, from a similar analysis, derive a figure closer to 40%. The two groups agree that the precise figure will be a function of the generator characteristics and the wind speed distribution function and this matter warrants further study.

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On an environmental model for the type Kimmeridge Clay

TYSON *ET AL.*¹ have proposed that Kimmeridgian coccolithic limestones were the result rather than the cause² of extreme anaerobic conditions analogous to those in the Black Sea today. Anoxic water columns develop where a salinity or temperature gradient causes density stratification which restricts circulation. However, anoxic mid-water columns develop in some areas due to high productivity in the euphotic zone³. If circulation was restricted, perhaps by topography, this process could go to extreme, therefore the suggestion of Gallois² cannot be discounted. The microlaminated marls associated with oil shales may form at maximum development of anoxic conditions. However, observations of major developments of the coccolithic limestones contradict this proposition for their origin. Also, carbonate will tend to dissolve below the O₂–H₂S interface⁴.

The Rope Lake Head Stone Band interbedded with the oil shales is bioturbated with *Rhizocorallium* and encrusted with oysters. The White Band contains distinct burrows at certain horizons and ripple cross-lamination. The evidence is conclusive, the major developments of coccolithic limestone occurred in aerated bottom water. Transpositional structures within the limestones indicate the substrate was unstable and this would account for the lack of benthos in places.

I believe the oil shales mark the maximum stand of the O₂–H₂S interface. Vertical movement of this interface could cause the lithological change clay–bituminous shale–oil shale, but dilution by terrigenous material can account for it equally well. In the Kimmeridge Clay both factors interact. The coccolith limestones accumulated when circulation increased. The previously anoxic water would supply concentrated nutrients, in particular HCO₃[–], and favour the propagation of coccoliths. This model accounts for incomplete cycles, such as, bituminous shale–coccoliths–bituminous shale and is more consistent with observational evidence.

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TYSON REPLIES—Irwin has proposed an interesting modification of the stratified water column interpretation for the cyclic sedimentation observed in the type Kimmeridge Clay. While we apparently

agree that the sequence clay–bituminous shale–oil shale represents a transition from aerobic to anaerobic bottom conditions (coupled with the progressive development of an O₂–H₂S interface in the lower part of the water column) our respective interpretations for the depositional conditions of the coccolith limestones are directly opposed.

On the basis of field observations Irwin claims that the coccolith limestones were deposited in aerobic bottom conditions when the dispersal of anoxic, nutrient-rich bottom water had promoted increased propagation of coccoliths. As I have only just completed a detailed examination of the sequence I must contradict Irwin's evidence: (1) The Rope Lake Head limestone, although bioturbated, is not oyster encrusted (and is not a true coccolith limestone). (2) Only a single poorly developed horizon of bioturbation occurs in the White Stone Band coccolith limestone, reflecting what was clearly a transient improvement in bottom oxygenation (for example, associated with limited advection due to a density current). (3) The White Stone Band coccolith limestone does not contain any evidence of bottom currents, but penecontemporaneous deformation structures do sometimes resemble ripples. (4) The coccolith limestones are devoid of benthos.

The remainder of Irwin's argument contains several inconsistencies. While solution of inorganic carbonates (such as, 'seekreides'¹) is an important process below the O₂–H₂S interface, it is not relevant to this discussion for coccoliths are at present day accumulating on the floor of the Black Sea² (despite pH values of 7.6 ref. 3). If the deposition of coccolith limestones were initiated by the dispersal of anoxic bottom water (coincidental with the development of aerobic bottom conditions) then: (1) There is no reason why 'varve-like' microlaminations should form. (2) Any microlaminations would have been destroyed by bioturbation (there is no *a priori* reason to suppose the substrate was unsuitable for benthos—especially when one considers the bioturbation in the White Stone Band). (3) This would contradict the geochemical⁴ and palynofacies evidence (ref. 5 and personal observations). (4) It would imply that the coccolith limestones should always be underlain by oil shales (that is, sediments representing anoxic bottom conditions) which they are not. (5) According to Gallois⁶ the decay of the resultant phytoplankton bloom would recreate anoxic bottom condition anyway. (6) A greater degree of lateral variation would be expected. Bottom water dispersal events are recorded in the stratigraphic record, but not by laminated coccolith limestones⁷.

Any belief in a stratification/O₂–H₂S interface model is incompatible with Gallois' original hypothesis⁶ which was an alternative to the restricted basin models.

If one does believe in stratification one should accept that with the onset of stagnation the O_2 - H_2S interface will inevitably rise to the base of the euphotic zone (where it will participate in the plankton dynamics of the basin) unless one of several external influences disrupts the system⁷. Much more convincing evidence is required if Irwin seeks to replace a model which has the weight of Quaternary-Recent modern analogues behind it.

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Calcium activation of the cortical reaction in sea urchin eggs

USING sea urchin (*Echinus esculentus*) eggs broken by shock discharge, or isolated egg cortices, Baker and Whitaker¹ found that about 1 μ M Ca was sufficient to activate exocytosis of half the cortical vesicles. They represent this figure as an order of magnitude lower than the figure we reported² (between 9 and 18 μ M) using *Lytechinus pictus* sea urchin isolated cortices.

However, there is no discrepancy between our results. We measured the level of calcium required for nearly complete (>95%) exocytosis, and we have recently obtained a more precise estimate of 12 μ M, determined by breaking the eggs open in the test solutions. From Baker and Whitaker's Fig. 2, they observe 95% exocytosis at a Ca concentration of 6 μ M ($pCa = 5.2$). Moreover, Baker and Whitaker derive an apparent stability constant³ from a Ca-EGTA binding constant⁴ (pK) of 11. However, in physiological media a lower binding constant is appropriate⁵. We assumed a pK of 10.7 in our calculations for our HEPES-buffered solutions, as this value was reported⁶ for similar phosphate-buffered solutions. This difference in binding constant accounts for the twofold difference remaining between our calculated Ca threshold for the cortical reaction and that offered by Baker and Whitaker.

The problem of selecting the appropriate binding constant for EGTA is a general one in studies of the role of calcium. We recently attempted to estimate the primary Ca-EGTA binding constant ourselves, using calcium-specific electrodes filled with Ca-DOPP mixed with

DOPP-n⁷. We could only obtain accurate measurements of calcium when the apparent binding constant of EGTA for Ca was reduced in PIPES-buffered low pH (6.5) and the monovalent cation concentration was kept to 0.13 M. We measured an apparent binding constant, $pK' = 5.3$, corresponding to a primary Ca-EGTA binding constant of $pK = 10.6$. Since high pH⁸ and high ionic strength⁹ are both reported to reduce the primary Ca-EGTA binding constant (but see ref. 10), it seems that even our estimate for the Ca threshold of the cortical reaction (12 μ M) is too low.

Baker and Whitaker also suggest that ATP is needed to maintain the cortical intracellular Ca store. We found that sperm, the Ca-transporting membrane ionophore A23187, and the parthenogenetic agent urea all release Ca from the same store¹¹. Quantitative estimates of the amount of Ca released^{2,12} compared to the cortical reaction threshold, suggest that the store is released into a small fraction of the cytoplasm at the cortex. As the store is replenished and can be discharged again about 45 min after release (when the cortical vesicles are gone), it is unlikely to be associated with the cortical vesicles themselves. The calcium store may reside in a subsurface endoplasmic reticulum, but this has not yet been observed in the appropriate cortical region. The calcium may be bound directly to the membrane instead.

We thank Sherwin Lee for help with the Ca-sensitive electrodes.

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BAKER AND WHITAKER REPLY—We are delighted that Zucker and Steinhardt find no serious disagreement between their results and ours.

It is unfortunate that one cannot be sure of the value of the free calcium concentration in physiological solutions buffered with EGTA to within half an order of

magnitude. As Steinhardt et al. pointed out¹ the most one can do is to specify total calcium, magnesium and EGTA concentration so that, should reliable affinity constants become available, the free calcium data may be recalculated. Although monovalent cation concentrations were not identical, a rather direct comparison of the results obtained on British and American urchins is possible because the calcium buffers used in our experiments² were made up in the same proportions of calcium, magnesium and EGTA as those specified in the study of the cortices of *Lytechinus*.

Much of the apparent discrepancy stems from Zucker and Steinhardt's somewhat peculiar definition of 'threshold' for cortical granule release as the calcium concentration giving release of 95% of the granules. Although the release of individual granules is an all-or-none process, our observations suggest that in a population of granules the rate of discharge is a smooth function of calcium concentration. Physiologically, it is likely that the abrupt onset of the cortical reaction can be attributed to the release of large amounts of calcium from a store³. In the presence of 5 mM ATP, a calcium test solution² which according to Steinhardt et al.¹ contains 1 μ M free calcium, initiates a cortical response which spreads as we have described and has discharged all the cortical granules within 2 min. At higher calcium concentrations the rate of discharge of the granules (expressed as a percentage of the total number) increases. A calcium activation curve obtained by scoring the proportion of granules remaining at 30 s has a similar half point to the data (Fig. 2a of ref. 2) for eggs subjected to high voltage discharge in solutions of different calcium concentration. Again, using the constant of Steinhardt et al.¹ the half activation point is about 3 μ M, although its position will depend on the time after addition of calcium at which the reaction is assessed. At its fastest, the discharge of cortical granules over the whole cortical fragment takes about half a minute. This is not dissimilar to the rate *in vivo*⁴. Without information as to the time of observation, the half point of activation in the cortices of *Lytechinus* cannot strictly be estimated, but might at its lowest be 6 μ M. At worst, as it is not clear whether concentrations of calcium less than 12 μ M cause any granule discharge in *Lytechinus*, there remains an order of magnitude difference between the two species in the lowest calcium concentration which has been demonstrated to produce a cortical response.

Although this difference in the affinity of the cortical reaction for calcium in the eggs of the British and American urchins may well have its explanation in species differences, or the uncertainties in free calcium concentration, our observations as to the critical importance of ATP in maintaining the properties of the

physiological reaction² suggest a less trivial explanation. As experiments with *Lytechinus* were performed in the absence of added ATP, the decrease in calcium affinity which results from removal of ATP in *Echinus* may well be the factor responsible for the apparent differences in calcium affinity in the two species.

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Fusion or lysis of vesicles by Ca^{2+} ?

GINSBERG showed¹ that sonicated phosphatidylserine (PS) vesicles in the presence of large concentrations of Ca^{2+} or Mg^{2+} did not retain sucrose and he concluded that the final structures had lost the form of closed vesicles. As such, he proposes the cation effect to be one of lysis and the Ca^{2+} -PS system to be an inappropriate one for the study of membrane fusion. It is not surprising that the final product of the PS-metal interaction cannot retain solutes such as sucrose, as the vesicles collapse and internal volume is lost². The PS membrane repeat determined by X-ray diffraction is 53 Å in 2 mM Ca^{2+} ; 67 Å in 10 mM Mg^{2+} (ref. 2) and 71 Å in 1 M Na⁺ (ref. 3). The term 'membrane fusion' refers to the formation of larger membranous structures by contact and mixing of the parent membranes. This is in contrast to the mixing of membrane lipids by diffusion of the components, as proposed for the dimyristoyl lecithin-dipalmitoyl lecithin system⁴. Recent experiments³ have shown that release of contents is nearly second order in vesicle concentration and is concomitant to aggregation, demonstrating vesicle contact in leakage experiments. That Ca^{2+} induces fusion, that is, mixing of membrane components, is indicated by the formation of large cochleate structures, which on addition of EDTA, become huge vesicles capable of entrapping large molecules⁵. Mg^{2+} alone is less effective⁶ but in its presence, only small concentrations of Ca^{2+} are required for obtaining larger structures^{2,3}. Similarly, in the dimyristoyl phosphatidylglycerol-dipalmitoyl phosphatidylglycerol vesicles⁷ and in phosphatidic acid-phosphatidylcholine vesicles⁸, Ca^{2+} induces mixing of the lipids, that is, fusion. Our view is that fusion requires a destabilisation of the membranes in contact until the joint membrane is arranged. Model systems are intended to be approximations to the *in vivo* systems. A definition of

membrane fusion as a process without leakage is too restrictive and so far no experiment has ruled out leakage in fusion events. In model systems better approximating the *in vivo* system, such as mixed PA/PC vesicles⁸, mixing and retention of contents has been demonstrated. The interactions postulated to occur between the Ca^{2+} and PS in the pure system are still relevant to the mixed systems.

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GINSBERG AND GINGELL REPLY—The comments of Nir and Pangborn stem from an inadequate definition of membrane fusion. They claim that fusion is equivalent to the mixing of components from two contacting parent membranes. However, physiological membrane fusion apparently involves the transfer of aqueous contents from one membrane-bounded compartment to another without spillage into inappropriate spaces, as seen in phagosome-lysosome interaction and secretion. Any artificial system should fulfil this additional criterion to be biologically relevant^{1,2}. Thus, the X-ray data cited by these authors³ seem to invalidate their PS system as a paradigm for membrane fusion: Ca^{2+} converts PS into a multilayer containing no removable water. Such collapsed multilayers could result from the lysis of closed membranous forms by the mechanism we suggest¹. The experiments described by Nir and Pangborn where loss of contents is reported to accompany vesicle aggregation may also be explained in terms of lysis: Ca^{2+} -induced vesicle rupture with loss of contents may provide the antecedent for immediate aggregation of the resultant membrane fragments.

Although Ca^{2+} is strongly implicated in biological membrane fusion⁴, there is no compelling reason to suppose that its action on dispersions of single acidic phospholipids resembles its interaction with mixed lipid membranes nor with the biomembrane systems in which fusion was first studied⁵. This point is underlined by the far greater Ca^{2+} sensitivity of natural vesicle fusion⁶. Gershfeld has recently shown⁷ that sonicated vesicles are in a metastable state at temperatures exceed-

ing the lipid phase transition temperature. Thus, addition of divalent cations to PS vesicle suspensions may merely trigger a return to equilibrium by a variety of unknown pathways.

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Some real communities are unstable

THE mathematical stability analyses of randomly constructed food webs of Pimm and Lawton^{1,2} have emphasised the destabilising influence of omnivory. Their examination of a number of real food webs³ seems to support their hypothesis that webs with many omnivores should be rare except in insect host-parasitoid systems. However, I analysed the real food webs cited as corroborative by Pimm and Lawton, that is, those of Askew⁴, Force⁵ and Richards⁶, and found that none meet the criterion for Lyapunov stability. The validity of the models and their inherent assumptions appears questionable.

All webs were analysed using the observed signs of interaction and Pimm and Lawton's constraints on the selection of random magnitudes for parasitoid-host and herbivore-plant interactions. Signs for variable interaction, species A and B each serving as prey or predator for the other, were arbitrarily assigned. To investigate whether the occurrence of self-limited species influenced stability, runs were repeated with self-regulation terms removed (all principal diagonal elements equal to zero). Finally, self-limitation was introduced at the lowest trophic level (plant), a criterion used by Pimm and Lawton in constructing their random webs. Analysis of Richard's web was performed twice, once with the parasitoid-host and again with vertebrate predator-prey constraints used for the predatory arthropods. In no case were any of 50 runs for either the original or adjusted webs stable.

I included only primary interactions in abstracting a matrix from Force's web. The excluded terms (dashed lines in Force's paper⁵) represent secondary interactions, such as the rejection of a potential host which has been previously

parasitised by another parasitoid. The existence of this exploitative competition, which has been reported for many parasitoids^{4,7}, implies that higher order interactions are common in parasitoid communities. However, higher order terms violate an assumption of the stability analysis.

If Lyapunov stability is a relevant feature of communities and extinction of some member(s) of each community is not imminent, there are several explanations for the observed discrepancy. Perhaps stable solutions exist, but have not been discerned, as randomly selected parameter values do not exhaust all possible combinations⁸, and the use of unequal constraints for different trophic levels prohibits tests of qualitative instability. Natural selection may drive a community towards a restricted region of parameter space where mathematical stability is realised⁹.

An alternative explanation is that the community models are inadequate. Coincident with Pimm and Lawton's predictions, the instability of these webs is apparently the result of high connectance (percentage of non-zero elements in the interaction matrix), reflecting the presence of many omnivores. In fact, both of Askew's webs include omnivores whose alternate prey occupy widely separated trophic levels. This is the most destabilising form of omnivory according to Pimm and Lawton. If these webs are in fact stable, perhaps the constraints on selection of interaction magnitudes are biologically unrealistic, as the other stability parameters (connectance, number of species) are observed quantities. It has been demonstrated that as the range of constraints is narrowed, implying lower interaction intensities, the probability of a random community exhibiting stability increases. The host switching of many parasitoids and the concomitant unequal use of alternate hosts suggests that a narrower range of constraints should be used for some of the parasitoid-host interactions.

The problems associated with the community models may involve more than just the determination of actual interaction intensities and their linearity. Two assumptions underly the models and the resultant stability analyses. First, communities are considered to be definable closed systems uninfluenced by immigration and chance extinction. However, real communities are dynamic entities. The delineation of ecologically meaningful boundaries of a community can be critical to stability analysis as some unstable communities are comprised of several stable subunits¹⁰. Furthermore, Levin¹¹ has demonstrated that immigration and spatial heterogeneity can profoundly influence community stability. The second assumption is that the interactions we are attempting to estimate, competition or predation, are responsible

for both the population dynamics of the species comprising the community and its organisation. Many predators, particularly parasitoids, can greatly influence the abundance of their prey as evidenced by successful implementation of biological control, but their role in community organisation is less clear. Similarly, the role of competition in structuring communities and controlling species abundances is a hotly contested issue^{12,13}. Therefore, in light of the current lack of understanding of the determinants of community organisation, I question the predictive value of community modelling and mathematical stability analyses.

I thank Daniel Simberloff, Stan Faeth and Donald Strong for their comments.

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LAWTON AND PIMM REPLY—

Auerbach has overzealously applied an extremely simple model; Lotka–Volterra equations do no more than capture the shadow of real biological interactions. We used such models to generate qualitative predictions¹, and deem it extremely unwise to attempt more quantitative fits to real webs. However, this does not mean that our hypotheses cannot be tested.

Our main qualitative prediction was that omnivory has a destabilising influence on food webs, the more so when predators are bigger and rarer than their prey, and have a large *per capita* effect on the things they eat. We were deliberately cautious in assessing this prediction, noting simply that it was 'encouraging' to find very complex webs in the real world in exactly those situations predicted by the models, that is amongst insect host–parasitoid interactions. More detailed analyses confirm that insect food webs do have significantly more omnivory than other webs². Indeed none of several interesting qualitative predictions yielded by simple Lotka–Volterra models^{3,4} are refuted by data from real food webs². To test our hypotheses further, we would prefer to see whether these qualitative predictions still hold when the major assumptions of the models are changed, rather than try to force unmodified Lotka–Volterra equations beyond their sensible limits. For example, if incorporating spatial heterogeneity reverses our predictions (making

omnivory easier to achieve in non-parasitoid than parasitoid webs), then our results are a nonsense. We doubt whether any of the refinements listed by Auerbach will have this effect.

Far from refuting our models, Auerbach's analysis confirms our conclusion that omnivory is destabilising: it is much easier to find stable solutions for simple webs than it is for complex ones. At present, we see no evidence for claiming that the majority of persistent natural populations are best described by model analogues that are inherently unstable^{5,6}. However, the detail needed to stabilise the models depends on the nature and rigour of the questions being asked. Auerbach suggests several possible reasons why his Lotka–Volterra models of real food webs are always unstable. Undoubtedly higher order interactions^{7,8} (which do not violate the assumptions of a local stability analysis, but make non-equilibrium behaviour difficult to predict), the precise choice of parameter values (we put webs into the correct relative ranking: the numbers themselves were guessed, not measured, and may well have been wrong in detail?) and spatial heterogeneity are all important. The effects of spatial heterogeneity, in particular, must be incorporated before models can accurately predict observed levels of host depression by parasitoids⁹. (For a wide range of models of varying levels of complexity, yielding insights with different degrees of rigour, see ref. 10.)

However, Auerbach's note is valuable in drawing attention to biological details that must be important in determining the fine structure of food webs. Viewing webs as static entities with fixed links is no more than a crude beginning^{11,12}. The concept¹³ of webs 'resonating' between different configurations, each of low complexity, may well provide a valuable theoretical starting point for more sophisticated analyses.

We thank Mike Hassell and Simon Rallison for comments.

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reviews

Basis for an ethical system

Stuart Sutherland

Beast and Man: The Roots of Human Nature. By Mary Midgley. Pp. 377. (Harvester Press: Brighton, Sussex, 1979.) £7.50

Beast and Man has been widely praised by the litterateurs who contribute to the book pages of the surviving intellectual weeklies. It is a long, rather rambling book, in which it is difficult to discern a clear connected argument. Its main aim seems to be to establish a secure basis for ethics. Mary Midgley argues that, like other animals, man has many inborn drives: these drives can only come to fruition within the context of a culture. She argues that what is good for man must depend upon the nature of his motives: moral reasoning consists of the attempt to harmonise conflicting emotions and to give priority to those which are most deeply ingrained in our nature. It will be a comfort to scientists that she believes the scientific study of human nature can assist in making ethical decisions by revealing more about man's true nature. A second theme running through the book is that we are part of the Animal Kingdom and of the Universe and that one principle of morality is that man should have respect for other animate beings and indeed for the inanimate universe.

In preparing the ground for her own thesis, she assails previous views of the nature of goodness, but she frequently seems to espouse the mistakes of which she accuses others. For example, she attacks Aristotle for arguing that the ultimate human value must be the development of that function which is unique to man and not shared by other animals. She rightly remarks that "it must be shown separately that this differentia is itself the best human quality, that it is the point where humanity is excellent as well as exceptional". Yet later in the book she writes: "Should we therefore say that everything we want is good? In a minimal sense this is right...but...we must go further...because of a competition amongst our various wants. What is good in a stronger, more considered sense must be wanted not just by someone's casual impulse, but by *him as a whole*" [her italics]. The meaning of "him as a whole" is obscure, but supposing

it were to turn out — as it might — that hatred for outgroups was an inborn human characteristic, she would seem to be committed to the proposition that it was thereby good. Edward Wilson has emphasised that the mechanism of evolution depends on the selection of genes that survive and she criticises him for erecting the survival of the individual gene into a principle of morality, but she herself seems to be committing the same fallacy in arguing that whatever is an essential part of human nature must be good.

When she wishes to recommend a given course of action she tells her readers that it is "natural". She believes that: "Respect for other forms of life is certainly a natural feeling. It is not a mere inclination, it is a feeling that we must not destroy certain things — and one that is not isolated, but forms part of our central system of standards". She provides no arguments to show that this feeling is any more natural than delight in wanton cruelty; nor does she provide criteria for deciding that something is natural nor even attempt any clear definition of what the word means. The fact that something forms part of the "central standards" of some people cannot automatically make it good: many societies have as part of their central system of standards a belief in the duty to avenge themselves and their families for slights.

Much of her book seems to be based almost on word play. For example, she insists that the word "rational" is used not merely to describe the behaviour of someone who makes the correct moves in solving intellectual problems, but also the behaviour of someone who acts consistently from a well harmonised set of emotions. Her point about the use of language is right, but it does not seem to advance her argument and it is not clear that it is any more difficult to harmonise a set of bad motives than a set of good ones.

She frequently embarks on arguments that look promising, but they tend to peter out. For example, she uses a cunning analogy to attack one of Wilson's arguments: he believes that understanding how ethical systems have evolved and how our reasoning about morality is controlled by the nervous system will throw light on the nature of ethics. She points out that

discovering how the workings of the brain enable us to do mathematics will not illuminate the nature of mathematics: to understand that we must understand the relationship between numbers, how mathematical proofs work, and the standards by which to judge the correctness of a mathematical calculation or proof. But she makes no attempt to explain what the standards are that are used in moral argument or decision making. It is easier to agree about the canons of mathematics than to resolve a disagreement about ultimate goals.

Although the book as a whole is both unsatisfactory and unsatisfying it does contain nuggets of robust common sense, many of which are well put even if they are not entirely new. For example, in arguing for the continuity of man and the Animal Kingdom, Mary Midgley points out that we often project our own evil on to animals and use a double standard in evaluating their behaviour and our own. It is unreasonable to despise the fox who kills hens for sport more than those who ride to hounds. Again, she points out that we cannot abnegate from instilling a culture and norms in our children — an adult who tries to adopt a non-committal position and tells the child he need not accept a particular ideal until he is old enough to judge for himself is in effect saying "I do not take this seriously and nor need you" and is likely to instil norms of "timidity, shiftness, and dilettantism". She proposes a sensible if slightly vague resolution of Wilson's dilemma over how altruism towards unrelated members of the same species may have originated: it may have been built on filial and parental instincts, and indeed affectionate gestures between adults mimic those made by parents to children and children to parents.

In view of her obvious sincerity and high-mindedness and the importance of the endeavour, it is a pity Mary Midgley has not produced a more cogent and carefully argued book, but most previous attempts to establish a secure basis for an ethical system have proved equally unsuccessful.

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Protein denaturation

Physicochemical Aspects of Protein Denaturation. By S. Lapanje. Pp 331. (Wiley: Chichester, UK, and New York, 1978.) £19.50.

THERE has been no significant, full-length review of protein denaturation since 1970. Although many fundamental principles had been laid at the time, there has been a steady advance in the detailed understanding of denaturation in the intervening years. Further, the biological and industrial significance of conformational change and stability is more widely appreciated today, and more chemists, biochemists and physicists are ready to devote time and energy to rigorous studies of the dynamics of protein structure. The field of protein denaturation has a distinguished history, starting with Ramsden's observation in 1902 that "a dead frog placed in saturated urea solution becomes translucent and falls to pieces in a few hours". Anfinsen's thermodynamic hypothesis of folding, the concept of limited pathways, quantification of protein stability, the hydrophobic interaction and prediction of tertiary structure all owe something to denaturation studies, and many of these areas are still under active study.

This monograph sets out to survey the physicochemical basis of denaturation and therefore leaves the reader to apply the fruits of this approach to his own field of work and interest. Methods appropriate for studying denaturation are described; in each case a self-contained account of the fundamentals is given, with examples taken from conformational studies on proteins and polypeptides and references to recent reviews for further detail. Techniques include hydrodynamic, optical, light scattering, NMR and hydrogen exchange, although calorimetry, surprisingly, considering the author's interest, is given only passing mention.

The largest section of the book comprises a survey of experimental results obtained with a range of perturbants including heat, pH change, urea and guanidine, inorganic salts, organic solvents, and detergents. This draws together a wealth of detail from the literature and enables the reader to compare the different and frequently rather specific effects of these modes of denaturation. The results are well illustrated with data from original papers. It is useful to have the literature presented objectively and without the constraint of a particular theoretical viewpoint.

The chapter on the thermodynamics of denaturation, an aspect to which the author has made significant contributions, contains a good discussion of the two-state hypothesis and an up to date account of calorimetric studies and of the thermodynamics of thermal denaturation.

Although it is mentioned in several places that the denatured states may differ depending on the mode of perturbation, I could find no discussion of the dependence of derived thermodynamic parameters on the definition of the denatured state. Also, the values of n in the relationship $K_D^1 = A c^n$ surely range wider than 12 to 20 — probably a misprint. The interpretation of biphasic kinetics of unfolding and refolding of proteins in terms of the proline isomerisation model is a particularly hot issue at the present moment and the short chapter on kinetics of denaturation provides a useful background to the various ways in which such experiments have been treated. This leads into a final chapter dealing more with interpretation of kinetic and thermodynamic experiments, particularly with respect to the mechanism of folding of globular proteins and the nature of the interactions of denaturants with proteins. As regards the latter, the author seems to favour the direct binding of urea molecules to a protein as a driving force in denaturation, and it is an interesting exercise to try and fit an alternative explanation to the results he quotes purely in terms of change in water structure.

This monograph is valuable as an unusually well organised survey of a massive literature. I found it stimulating, not because of strongly argued personal ideas, but because of the creative apposition of the different experimental approaches and interpretations which have been used in this field. It is, however, encouraging to find that his thermodynamic hackles are capable of being roused when his basic convictions are challenged (see p295). This review is valuable also in that the author emphasises the complexity of deriving and assigning thermodynamic parameters from systems as complex as proteins in multicomponent solvents.

The book is well produced and well illustrated. The index contains the reviewer's favourite enzyme but omits "two state transition". One is enormously impressed by the feat of writing (over 300 pages) in what is, to the author, his third language. Only occasionally is there a misplaced and, very occasionally, a misleading nuance. Most of these are so obvious that in more happy and responsible days one might have expected the publisher to have brought them to the author's attention. The duplication of equation 2-26 and the confusing layout of Table 4.4 also fall into this category. Nevertheless, this is a monograph that will be an essential reference work for all those concerned with the stability and dynamics of proteins and provides excellent value for money.

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Semiconductors textbook

Semiconductors. Second edition. By R. A. Smith. Pp. 523. (Cambridge University Press: Cambridge, New York and Melbourne, 1978.) Hardback £27.50; paperback £8.95.

THE publication of a second impression of any major textbook provides an indication of its value: its publication twenty years after the original edition in a field that had been developing very rapidly over most of this period gives further proof of the lasting value of the treatment which has not dated significantly. When R. A. Smith's book appeared in 1959 it provided the most detailed text on the then new and rapidly developing subject of semiconductor physics, with special emphasis on transport and optical properties. Now that the subject has reached what might be called mature age, the new edition is to be welcomed as a continuation of this tradition, with some significant modifications in order to introduce new topics and to update the bibliography. With the avowed intention of keeping the total volume constant, this was not an easy task, and the author may be congratulated on having, on the whole, succeeded rather well. As one part of the adopted strategy the reader is increasingly referred to the other major text by the same author, *Wave Mechanics of Crystalline Solids* (Chapman and Hall: London, 1961) and this means that the student will find the use of two textbooks more imperative than with the original edition.

The layout of material departs significantly from the original, partly to accommodate the additional subjects and partly to streamline the treatment. The first four chapters on elementary properties, energy levels, impurities and carrier concentration remain virtually unchanged. The original very extensive chapter on electron transport has been altered in several respects, one of which is the omission of the Boltzmann equation as the basis for the treatment of transport theory—in this reviewer's opinion, a retrograde step at the level aimed at in the book. Scattering mechanisms and high field effects are relegated to separate chapters and a new section is introduced on conduction at very low temperatures. Optical and high-frequency effects have been significantly expanded, the behaviour in high electric fields has been coupled with that in high magnetic fields to take account of recent advances, including the important transferred electron phenomena and carrier freezeout.

The original chapter dealing with the determination of semiconductor properties has been removed; and the chapters describing the properties of elemental and compound semiconductors have been shortened into one, no doubt on the reasonable basis that the volume of information on properties of semiconductors is so vast that it requires reference to specialist texts for any but the most elementary data. Instead, we have a short chapter on band structure and the effective mass approximation describing some recent developments such as the LCAO and the k.p methods and the pseudopotential. Another new chapter on "Some special topics" deals with excitonic molecules, electron-hole droplets, polarons and polaritons, tunneling in heavily doped materials, and the tunnelling spectroscopy and laser action in semiconductors, among others. The book closes with another new chapter on amorphous semiconductors providing a brief outline of this topic on a mainly descriptive basis.

The bibliography has been brought up to date in some of the more important respects but there is no pretence of giving complete listings which would become rapidly dated.

The overall impression is a very favourable one: this is still one of the most authoritative and comprehensive texts on this subject in one volume,

with a very detailed treatment of transport and optical properties and with a less detailed discussion of the band structures and of other physical properties of semiconductors. There is no significant discussion of any semiconductor devices, again on the reasonable assumption that this would make the text either much too long or too superficial.

One criticism of the presentation is the relatively inefficient use of diagrams: the information content of many of these is low for their size and some are too closely similar to represent real value—for example, Figures 13.3 and 13.4 giving the temperature dependence of the forbidden gap in germanium and in silicon. More economy in this respect would have permitted the inclusion of a wider range of information.

These are, however, minor criticisms of an otherwise excellent text which will continue to provide the basis of both undergraduate and postgraduate lecture courses and will represent a useful general reference text. The price, especially of the paperback edition, is very reasonable and the presentation is excellent throughout.

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Standard reference work on the plastids

The Plastids: Their Chemistry, Structure, Growth and Inheritance. Second edition. By J. T. O. Kirk and R. A. E. Tilney-Bassett. Pp. 960. (Elsevier/North Holland/Biomedical: Amsterdam, New York and Oxford, 1978.) Dfl337; \$149.75.

THE first edition of *The Plastids* has become something of a standard reference book. Because of the great advances which have taken place since this first edition was published, it was already beginning, after only a decade, to show its age. The authors have therefore undertaken a complete revision of their work, and the new enlarged edition contains a vast amount of additional information not earlier available. Each chapter now begins with a useful summary which allows the reader to find his way quickly and easily through the book.

The present volume attempts to cover the whole plastid literature, and

therefore necessarily goes over much of the same ground as previously. The text is first class and references to new work are included as appropriate. The figures and diagrams contain much new material clearly set out. But I was greatly disappointed by the re-use of many old electron micrographs; in view of the rapid advances which have taken place in electron microscopical studies of plastids since 1968 I suspect that many of the authors credited with the pictures used would have welcomed the chance to offer new micrographs of material treated by more modern methods of fixation. The quality of printing of the micrographs, in spite of the glossy paper used and in view of the remarkably high price of the book, is generally poor and indistinct and many of the pictures lack the contrast which the originals undoubtedly possessed. The pictures carried forward from the first edition seem to have suffered most in this respect.

Part I, *Chemistry, Structure and Function of Plastids*, is greatly enlarged and now comprises chapters 1–12 (pp.1–250), an increase of 160 pages. This part of the book will be of great value to someone wishing to have a

good general summary of the properties of chloroplast proteins, lipids and pigments and an account of the fine structure of the different plastid types. There are also excellent short chapters on chloroplast permeability and photosynthesis. Dr Kirk is to be congratulated on the excellence of the summary of the new biochemical material and the development of ideas which he presents in this section.

Part II discusses the "Inheritance and Genetic Behaviour of Plastids" and is written by Dr Tilney-Bassett (chapters 13–22, pp.251–521). The form of presentation has obviously been completely rethought. New material has been incorporated, some old material discarded and a major rearrangement has been carried out. The new data on plastid mutagens and the causes of mosaic variegation are particularly valuable and the problem of sorting out of plastids is examined well. The origin and stability of chimaeras is discussed adequately, although the description demands a prior knowledge of the ontogeny of the shoot apex. The sections dealing with the reasons for the stability of periclinal chimaeras and for the existence of mericlinal chimaeras are acceptable but are sufficiently important to warrant a more extended treatment in the context of a discussion of plastid behaviour in higher plants. However, an expert who already knows the sort of details he is looking for is very likely to find them in this as in other sections, and the comprehensive subject, taxa and author indexes, will guide him well.

Chapter 22, which concludes Part II, is a completely new one which describes plastid inheritance in *Chlamydomonas*. It is a particularly helpful chapter and brings into focus the insight which the use of a unicellular plant can give to critical studies on extra-nuclear genes. As *Chlamydomonas* can be grown in accurately controlled conditions, it can be conveniently subjected to precise experimental manipulations; this is of particular importance in studying the expression of such genes.

Part III, written by Dr Kirk, examines the "Biochemical Basis for Plastid Autonomy and Plastid Growth" (chapters 23–28, pp.525–862). While keeping to the same general form of the first edition, it includes a mass of new information. For example, the section on biosynthetic capabilities now runs to 100 pages against a previous 30, an indication of an area of most rapid development in the past decade. Throughout Part III great care has been taken to ensure a good balance in the presentation. The whole topic of chlorophyll synthesis has now been assembled in one unit in the new volume, as opposed to the scattered treatment it received in the first edition, and de-

tails of modern work on the synthesis of δ -aminolaevulinic acid and its conversion to protochlorophyll are included. In the past decade much information on nucleic acids and protein synthesis has been obtained and this is well discussed in chapter 26. The section on plastid development (chapters 27 and 28) has been expertly updated.

Part IV (chapter 29, pp.875–894) gives us the benefit of the authors' joint views of the directions in which future research may be expected to proceed. The coverage of references generally is good up to 1976, but the immense work of printing such a large volume means that the literature between 1976 and 1978 (date of publication) has of necessity had to be largely ignored. It is therefore satisfying to note that the literature already contains papers on several of the topics outlined jointly by the authors in

Part IV.

This edition of *The Plastids* is more readable than the earlier edition, but it is definitely not for beginners. However, workers in this very wide field will find an immense store of information and references to the literature. For such a large book there are remarkably few printing errors and grammatical inexactitudes. The efforts of revising *The Plastids* has been well worthwhile; the volume is very impressive. So too, unfortunately, is the price, which will put it beyond the grasp of all but the most dedicated of research workers. It will, however, find a proper place on library shelves in departments of biochemistry and plant sciences throughout the world.

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Techniques at low temperature

Experimental Techniques in Low-Temperature Physics. Third edition. By G. K. White. Pp. 331. (Clarendon, Oxford University Press: Oxford 1979.) £15.

GENERATIONS of graduate students (including the present reviewer) were brought up on earlier editions of Guy White's book, which for years remained the only available supplement to the unwritten body of lore handed down by one's predecessors and supervisor and colleagues. It is a detailed exposition of the how of experimental physics at low temperatures: how to attain the low temperature, and how to measure it when you have got it; not only how to set about designing cryogenic apparatus, but also how to translate the design into a functioning and (one hoped) leakless reality. The third edition has been reworked more thoroughly than its predecessor, and is the better for it. Some of the less used sections have been pruned to make way for new material reflecting the growing emphasis on work below 1 K, and the whole book has generally been brought up to date.

The eleven chapters deal in succession with the production and storage of liquefied gases, heat exchangers, thermometry, heat transfer, temperature control, cryostat design, cooling with ^3He , adiabatic demagnetisation, vacuum techniques, and with the physical properties of solids at low temperatures. There are substantial appendices giving tabular data on: vapour

pressures of cryogenic liquids; platinum resistance and thermoelectric thermometry; relevant physical properties of elements at room temperature; electrical resistivity; and on the thermal contraction and thermal conductivity of typical constructional materials. There is an extensive (and international) list of suppliers of the cryogenic equipment, components and materials referred to in the text.

White's book no longer stands alone, of course; there are also, for example, the admirable little text *Low Temperature Laboratory Techniques* by A. C. Rose-Innes (English Universities Press: London, 1973), as well as the excellent but more specialised works by O. V. Lounasmaa (*Experimental Principles and Methods Below 1 K*; Academic: London, 1974) and by D. S. Betts (*Refrigeration and Thermometry Below One Kelvin*; Sussex University Press: Brighton, UK, 1976); but it probably remains unique in the amount of useful practical detail which is included, and in covering so wide a sweep of low temperature physics and engineering.

It is a pleasure to welcome the third edition of *Experimental Techniques in Low Temperature Physics*, and to commend it to a new generation of research workers. It is readable, it is packed with ideas and information culled from laboratories all over the world, and it has an extensive and up-to-date bibliography. It is a book which every designer or user of low temperature apparatus will wish to have at his elbow.

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